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BIOLOGICAL BULLETIN

THE LIFE HISTORY OF *PLANARIA VELATA*.

W. A. CASTLE.

Previous investigators working with a common flatworm, *Planaria velata,* have observed that the ordinary type of life history of the species is an entirely asexual one (Child, '13). Following the emergence of the young animals from cysts is a period of growth which is followed by processes of histological degeneration and loss of internal structure as soon as the animal has attained a certain size. This is followed by fragmentation of the worm and the encystment of the fragments. The external aspects of this process have been mentioned by Stringer (Stringer, '09) and more fully described by Child (Child, '13). Observations on the histological details of the process of degeneration have been made by Alexander (Alexander, '26). Our observations confirm those of Alexander in regard to the details of this process.

From the encysted fragments new worms are reconstituted and emerge from the cysts after a period of from two weeks to several months. The time necessary for complete reconstitution is dependent upon the conditions to which the cysts are subjected. Asexual reproduction may continue in this way for an apparently indefinite number of generations (Child, '14) and until comparatively recently has been the only observed method of reproduction for the species.

Under certain conditions in nature *Planaria velata* has been observed to undergo a life history and course of development entirely different from the asexual one mentioned above. Accompanying growth there has been the differentiation of sexual structures. Under the most favorable conditions sexual development has been completed and the sexually mature worms have copulated and laid eggs. Although none of these eggs laid in the laboratory hatched there is no reason to doubt that they would

under favorable conditions develop after the manner of those of other planarians; however sufficient material has not yet been available to make possible a study of the embryonic stages.

Following sexual maturity or with the advent of unfavorable conditions before sexual differentiation is complete, it has been observed that the worms undergo a histological degeneration similar to that which precedes the fragmentation of an asexual stock. This may at first involve only the structures associated with the reproductive system. In this event the worms return to the asexual state and a period of growth may ensue before the ultimate degeneration that leads to fragmentation and encystment. Under conditions that are more decidedly adverse to sexuality a degeneration of the other internal structures may accompany that of the sex organs, and fragmentation and encystment will follow without any intermediate period of asexuality and growth.

It has been found that the life cycle of *Planaria velata* may be altered and controlled by subjection to the proper experimental conditions. This is true not only of the asexual cycle but of the sexual cycle as well. In nature the conditions bringing about encystment are a period of good nutrition and growth accompanied by an increase in the temperature of the water. Child observed that by partial starvation the onset of fragmentation and encystment might be warded off indefinitely. Worms were thus maintained at approximately the same size and in approximately the same physiological condition for more than two years (Child, '14).

Keeping the worms at a lower temperature has been found to prolong the period of growth that precedes encystment but not to inhibit encystment for an indefinite period of time. At room temperatures (18–20° C.) worms will cease feeding and proceed towards encystment when they have attained a length of twelve or fourteen millimeters, whereas worms kept in an ice box at 8–10° will attain a length of eighteen or twenty millimeters before the onset of fragmentation. Eventual encystment however is the result always to be expected when the temperature remains constant. Conversely it is found that at temperatures higher than normal worms can be induced to encyst at a much smaller size. The approach of encystment can be detected in advance by

the cessation of activity, loss of pigmentation, and the disintegration of the pharynx. The fate of worms in such a condition can be altered by manipulation of the temperature to which they are subjected. A rise in temperature will hasten the onset of fragmentation; lowering of the temperature will retard it. In some cases worms with degenerated pharynges have been prevented from encystment by a change in temperature from that of the room to 8°. These worms have regenerated pharynges, acquired more pigment, and become perfectly normal asexual worms at the lower temperature.

Sexuality has never been observed in specimens of *Planaria velata* collected from temporary pools or ditches. It seems quite probable that conditions in that kind of habitat are never favorable to the development of sexual structures.

In a spring at Valparaiso, Indiana, *Planaria velata* has been found to occur at all months of the year. During October, November, and December the worms are increasing very rapidly in numbers and in size. These worms presumably had their origin in cysts formed by the fragmentation of worms as the water became warmer the preceding spring. In the latter part of November and through December all of the worms of a length of eight to ten millimeters or over show a very distinct genital pore. Microscopical examination shows testes and ovaries are present. The height of sexual development is reached by the latter part of December. The largest worms collected then show not only a complete development of ovaries, testes, and atrial organs, but in some of them the vasa deferentia are well developed and packed with spermatozoa.

From mid-winter on there is a decrease in the number of sexual individuals as well as in the number of worms to be found. By the middle of March there is no trace of sex organs in any of the worms collected. Through the months of April and May the worms decrease greatly in abundance, the larger ones undergoing fragmentation and encystment and the smaller ones remaining, so that during the summer months only a few small or medium sized asexual worms are found.

The most evident variable factor that might be effective in influencing the sexual cycle in this location at Valparaiso is seen at

once to be temperature. During the months of September and October when the small worms are emerging from cysts there is a gradual fall in the temperature of the water. The maximum reached during the summer is about 12–13° C. By the end of October this has fallen to 8–9°. The minimum is reached by the end of December, and at that time completely differentiated sexual worms have been collected from beneath the ice where the water is no warmer than 5°. During late January and early February there is a pronounced decrease in the number of sexual worms before there is any appreciable rise in the temperature of the water. During the spring there is a rise in water temperature accompanied by the total disappearance of sexual structures. The majority of the worms fragment and encyst during these months of rising temperature; some however persist through the summer as asexual worms.

Experiments have been carried out to determine the extent to which sexuality is influenced by changes in temperature. First it is noted that when sexual worms are brought from their natural habitat and placed in the laboratory at a temperature of 18–20° there is a quite rapid disappearance of sexual structures. This occurs irrespective of food conditions. By the end of two weeks ovaries, testes, and atrial organs have completely vanished. This is followed at once by the fragmentation and encystment of all the larger worms. Medium sized and small worms become apparently normal asexuals, but if fed will grow and undergo the usual precystic changes as soon as they have attained a sufficient size.

The history of the majority of sexual stocks placed at a temperature of 8–10° is not different from this, although if the animals are not fed very few of them will encyst unless they are exceptionally large. The disappearance of sexual structures is much retarded—testes and a genital pore may remain evident for from four to six weeks after the date of collection. With the possible exception of one lot of worms further sexual differentiation has never been observed after the time of collection. In this single case worms which were exceptionally large and well differentiated sexually were isolated at 8° at the time of collection. Three eggs were laid by these worms within twelve days of the time of isolation. Subsequently to this the worms lost all sexual structures

and eventually fragmented and encysted in the usual manner. It is seen from these experiments that low temperature retards the disappearance of sexual structures once they are developed. We may now consider the possibility of experimentally developing sexual worms from an asexual stock by the manipulation of the temperature to which the worms are subjected.

That there might be no question of a genetic difference of strains producing sexual and asexual worms, stocks having their origin from cysts have been taken as the starting point of these experiments. Such worms when fed at room temperature have invariably undergone a period of asexual growth and eventually encysted without any suggestion of approaching sexuality. At temperatures ranging from 4 to 12° the result is the same if the worms are placed at that temperature soon after emergence from cysts and the temperature is maintained fairly constant throughout the period of growth. As might be expected growth at low temperatures is much less rapid and the worms attain a much greater size before encystment. These experiments show that sexuality is not to be expected at either high or low temperatures as long as the temperature is constant throughout the life of the animals.

Asexual worms raised to a length of ten or twelve millimeters at room temperature and then subjected to a temperature of 8–10° have repeatedly been found to show indications of approaching sexuality. In such worms ovaries and testes in advanced stages of gametogenesis have been found within two weeks of the time of the change in temperature. Atrial organs have been practically completely developed. In all of these cases the worms have been so small that complete sexual development was not expected. In none of the experiments resulting in the development of sexual worms have the animals been fed during the time while the sex organs were differentiating. It has been observed that with the resumption of feeding there is at first a slight further development of the sex organs that is followed by their degeneration and the return of the worms to the asexual condition. A change from room temperatures to temperatures of from 3 to 8° has not been found effective in bringing about sexual development. Such experimental stocks have continued slow asexual growth when fed

but have shown no development of sexual structures. It is apparent from the results of these experiments that temperature is an important factor in the control of sexuality in Planaria velata. From observations of the conditions under which sexuality is known to occur in nature it is probable that the rate of growth is also important. Experiments along these lines are being continued.

Histological studies have been made upon the differentiation of the sexual structures as well as upon the anatomy of the reproductive system of sexually mature worms. Other studies have been made upon the degeneration of sexual structures and the changes that accompany and precede either fragmentation or a return to the asexual condition. The structure of encysted fragments and the process of reconstitution within the cyst have been made the subject of further histological investigation. The encysted fragments consist of a layer of epithelial cells forming a sac which encloses a mass consisting largely of globules of fats, proteins, etc., but containing a few scattered nuclei. Some degree of protoplasmic continuity evidently exists throughout this mass, for in the process of reconstitution the pharynx arises from the middle of the mass at a considerable distance from any part of the wall. The region of the developing pharynx first becomes distinguishable as an area containing rapidly increasing numbers of nuclei. It has not been determined whether the axis of the new worm is a result of the gradient present in the old worm which has persisted through the processes of fragmentation and encystment or the result of a new gradient that has arisen because of conditions to which the cyst is subjected.

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THE DEVELOPMENT OF THE SOLDIER CASTE IN THE TERMITE GENUS *TERMOPSIS*.

HAROLD HEATH AND BLAKE C. WILBUR,

STANFORD UNIVERSITY, CALIFORNIA.

As is well known to the student of the subject, two theories have been proposed to account for the origin of termite castes. According to the first view the young are all alike at the time of hatching and subsequently are modified by extrinsic factors. The second view supports the claim that even before hatching two castes at least, the reproductive and worker or the soldier, can be distinguished by differences in certain of the internal organs. In an effort to determine which of these two theories is the correct one it appeared that the most favorable line of approach to the solution of the problem is through the study of recently established colonies of *Termopsis* where all of the young belong to the soldier caste. With such material the junior author confined his investigations to *T. angusticollis,* while the senior author directed his attention to *T. nevadensis.* As a result the number of molts has been established, the characteristics of each instar have been determined and a comparison of the two species at the close of our independent investigations indicates that the history of both species is, in all essential particulars, the same.

The number of young appearing in a colony varies according to the nature of its surroundings. Primary royal pairs, occupying burrows in very damp situations, have been found without either eggs or young at the end of eleven months. On the other hand in what appear to be optimum conditions there may be as many as twenty-one individuals of various instars, including one or two fully developed soldiers as well as several eggs in various stages of development. This being the case, a section of the pine belt along the southern shore of Monterey Bay was kept under observation, since the conditions throughout were not only fairly uniform but highly favorable as well. Records kept of census counts in this district during a period of twenty years may there-

fore be considered to indicate with a fair degree of accuracy the rate of increase from year to year. In 19 nests, examined in July and August (the months when colonies normally are established), the number of individuals, not including unhatched embryos, varied from 2 to 17. These probably belonged to communities not more than one year of age. In 16 other associations the number ranged from 31 to 47. Since these were collected during the late summer, and no intermediate numbers connected them, it is reasonable to conclude that these larger colonies were two years old. In 11 cases the membership ranged from 97 to 121; these, it is believed, are three years of age. Finally there were 14 colonies numbering 327 as a minimum and 497 as a maximum—probably a four-year development.

Whether these figures are strictly correct or not, it is certain that in the early years of colony development the rate of increase in the number of offspring of *Termopsis* is comparatively slow. Even in old colonies, where the royal pair is surrounded by a thousand or more descendants, the egg tubes never become so numerous that they reduce the queen to an inert reproductive sac as in the case of certain tropical species. Hence it is safe to assume, on the basis of extended observations, that a community with 450 members is certainly three years of age if not four as the above figures indicate.

As previously mentioned, no individual with wing buds has been discovered in young colonies. To be more explicit none are known to appear before the members of a community total approximately 450. In the case of *T. nevadensis,* for example, there were two nymphs of this type in a colony numbering 454 individuals, 6 were in another colony of 467 members, 7 in one with 497 and 39 in one with a total of 490. Beyond this point the number of winged individuals mounts fairly rapidly until in old colonies, judging from the extent of the burrows as well as the size of the population, they may outnumber those of the soldier caste. In the case of *T. angusticolis* the same feature exists, but from data in hand the winged individuals put in an appearance at a somewhat later date when the number of inhabitants is nearer 500.

Before entering upon a detailed description of the development

of the soldier caste it is of importance to note that practically all of our observations rest upon the study of small colonies which never exceeded a hundred individuals. The size of the burrows they inhabited also gave evidence of comparatively young communities. Furthermore the brains of nearly two hundred individuals, chiefly in the first instar, were carefully measured and the plotted results showed the same type of variation curve as did the head width measurements. About fifty gonad measurements were made which likewise showed the same type of variation as width of head and brain. These facts, coupled with the entire absence of any insect which showed even a trace of wing buds, renders it perfectly certain that the following observations relate solely to the soldier caste.

The newly laid eggs of *T. nevadensis* are approximately 1.4 mm. long by 0.57 mm. in greatest diameter, while those of *T. angusticollis* average 1.55 mm. in length by 0.58 mm. Diameter. This remains true even of those colonies where the royal pair is accompanied by hundreds of descendants, and doubtless are provided with a relatively generous food supply. As development advances all eggs increase in size at practically a uniform rate until at the time of hatching the eggs of *T. nevadensis* are approximately 1.5 mm. long by 0.6 mm. in diameter, and those of *T. angusticollis* are on the average 1.7 mm. in length by 0.68 mm. in diameter. In certain exceptional cases these lengths may be exceeded by as much as thirteen per cent., though this is generally accompanied by a proportional decrease in diameter.

Recently hatched individuals of both species are found to have the mid-gut filled with air. Whether this is swallowed or is secreted by the intestinal wall has not been determined, but it is reasonably certain that the resulting increase in body size enables the young insect to burst the egg membrane and escape. In a relatively short time the air disappears, and in several cases it was found that shortly after this stage the young are fed either by the royal pair or by some of the attendant older offspring.

Correlated with the fairly uniform size of the eggs of each species there is a corresponding uniformity in the dimension of the newly hatched young. This condition of affairs however usually undergoes a rapid change with the distention of the ab-

dominal wall due to food in the digestive tract and the development of the fat body, and in the later stages of this first instar, when the insect is about to molt, the body occasionally may become even larger than the smallest individuals of the succeeding instar. Body length, therefore, is not an absolutely dependable criterion for the determination of a given instar. Considerably better is the customary method of determining instars on the basis of antennal segments. This likewise is not always a trustworthy guide, especially in the later stages; but it has been found that where these antennal joint counts are combined with the measurement of the transverse diameter of the head the difficulties of distinguishing the various stages practically disappear.

In recently hatched young of *T. nevadensis* the head width averages 0.648 mm. on the basis of 182 individuals taken from 25 colonies the minimum being 0.58 mm. and the maximum 0.67 mm. The number of antennal joints is usually thirteen though twelve may occur. In some specimens this variation may appear on opposite sides of the same individual. The long third joint appears to be unsegmented, but in specimens treated with caustic potash and stained with magenta this segment is found to be subdivided into two incipient segments of which the distal one is the smaller. The first instar in the case of *T. angusticollis* is practically a duplicate of the same stage in *T. nevadensis*. The transverse head diameter, however, is somewhat greater. In 116 individuals from twenty-four separate colonies the maximum width was 0.81 mm., the minimum 0.74 mm. with an average of 0.7 mm. The number of antennal segments is fourteen.

The distribution of the spines over the body of recently hatched individuals in young colonies of *T. nevadensis* is shown in Pl. I, Fig. 1; of *T. angusticollis* in Pl. II, Fig. 7. Even in the oldest communities, headed by numerous substitute royal insects, there are no appreciable differences where individuals of the first instar are concerned. In other words the newly hatched young of each species are alike externally whether they belong to a recently established community or one that has existed for several years. An exception to this rule may be noted in the case of young hatched in colonies headed by old primary royal pairs or by substitute royal forms. In such cases the spines show the same gen-

eral arrangement as in younger colonies, but they are relatively longer, more slender and more flexible. This feature tends gradually to disappear as development progresses, yet spines of this character can be found to persist even into the penultimate instar.

Measurements of 192 individuals in the second instar, in the case of *T. nevadensis,* possessed a head width of 0.77 mm. in 13 specimens 0.86 mm. in 71 examples, 0.92 mm. in 78 individuals, 0.96 mm. in 28 others while 2 measured 1.0 mm. From among these 50 individuals were selected, treated with caustic potash, stained with magenta and compared. In every instance there were fifteen antennal segments; and the distribution of the spines over the body closely conformed to plan represented in Pl. I, Fig. 2. In the case of *T. angusticollis* the average head width amounted to 1.026 mm. with the usual range of variation. The number of antennal joints was sixteen; otherwise in its general proportions and distribution of spines the individuals of this instar closely resemble those of the same stage in *T. nevadensis* (Pl. II, Fig. 8).

Out of 117 individuals of *T. nevadensis* in the third instar the average head width amounted to 1.22 mm. The number of antennal segments was seventeen in 48 out of 50 specimens, the exceptions being due to the lack of the normal division between the third and fourth joints. The general appearance of the insect at this stage is represented in Pl. I, Fig. 3. In regard to *T. angusticollis* the average head width of individual in the third instar amounts to 1.3 mm. The number of antennal segments is almost invariably eighteen. Further details are shown in Pl. II, Fig. 9.

The fourth instar in *T. nevadensis* is characterized by an average head width of 1.65 mm. on the basis of 161 individuals. In the majority of instances the number of antennal joints was nineteen although 11 out of 50 had developed eighteen on one or both sides. For the distribution of the spines see Pl. I, Fig. 4. Comparing the same stage in *T. angusticollis,* the average head width amounted to 1.8 mm., and with few exceptions the number of antennal joints was twenty. Other external features are represented in Pl. II, Fig. 10.

The average head width of 136 specimens in the fifth instar was 2.35 mm. in the case of *T. nevadensis.* The number of anten-

nal segments varied from nineteen to twenty-one. In specimens of *T. angusticollis,* in the same stage of development, the transverse head diameter was 2.56 mm., and the number of antennal segments was twenty-two in the greater number of instances, although twenty or twenty-one was not an uncommon count.

Fully developed soldiers in recently established colonies are subject to a wider range of variation than is the rule regarding any other instar. In some instances the head width is but slightly greater than that of individuals in the penultimate instar; and the number of antennal joints may be that of the foregoing stage or even less, occasional insects having but nineteen segments. The average head width of 26 soldiers of *T. nevadensis* was 2.64 mm., while 18 soldiers of *T. angusticollis* measured 2.96 mm. in transverse diameter. The number of antennal joints varied from twenty to twenty-three or even twenty-four.

From the foregoing data it becomes evident that the two species under consideration are structurally closely related, and in each instance their development follows essentially the same path. Yet they have never been found to interbreed in a state of nature, and everywhere each colony of either species invariably appears to maintain its integrity. The number of antennal joints in the same instar is, with rare exceptions, one more in the case of *T. angusticollis,* and its greater head width is also a distinctive feature, so much so in fact that even without the royal pairs the two species can be distinguished without much difficulty. It may be added that the arrangement of the spines over the body has not been found to be of much diagnostic value even where the various instars are involved. Each additional molt adds to the number of bristles, but their location is much the same in both species.

As noted previously, this study deals only with the development of the soldier caste in relatively young colonies. In older communities, where the reproductive caste has put in an appearance, the history of the soldier is somewhat more complicated. To what extent this is true can only be determined on the basis of much more extended observations, including experimental work, measurements of head, brain, gonad and perhaps other anatomical characters, a study that is well under way with a strong probability that some of the more important problems can be solved within the next few months.

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PLATE I.

Explanation of Figures.

Successive stages in the development of the soldier caste in a young colony of *Termopsis nevadensis*.

FIG. 1. Newly hatched young; head width 0.65 mm.
FIG. 2. Second instar; head width 0.96 mm.
FIG. 3. Third instar; head width 1.0 mm.
FIG. 4. Fourth instar; head with 1.73 mm.
FIG. 5. Fifth instar; head width 2.18 mm.
FIG. 6. Fully developed soldier; head width 2.6 mm.

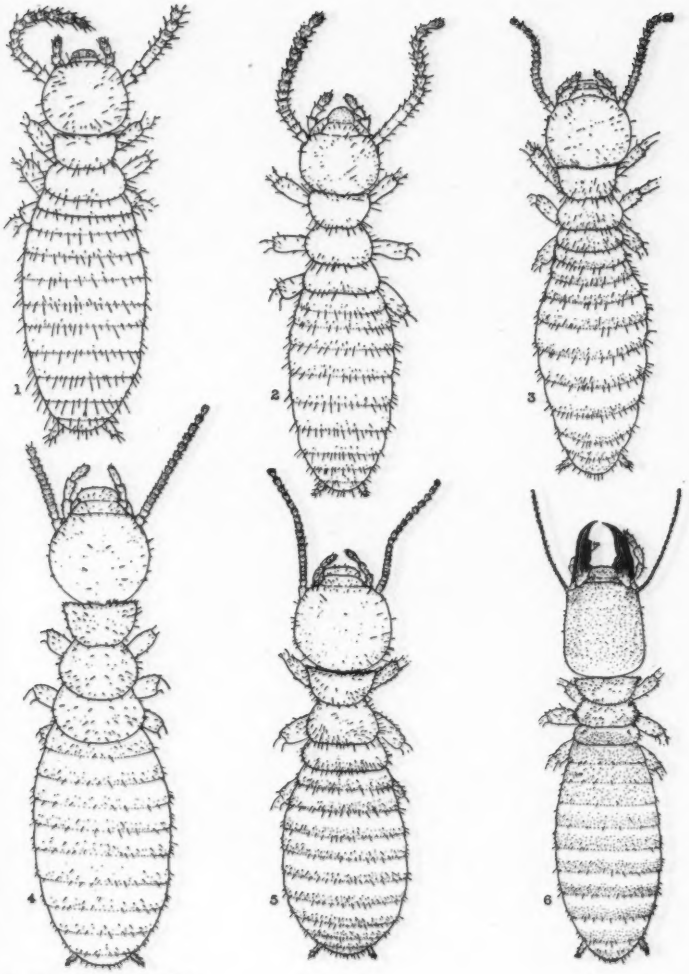

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PLATE II.

Explanation of Figures.

Successive stages in the development of the soldier caste in a young colony of *Termopsis angusticollis.*

FIG 7. Recently hatched young; head width 0.72 mm.

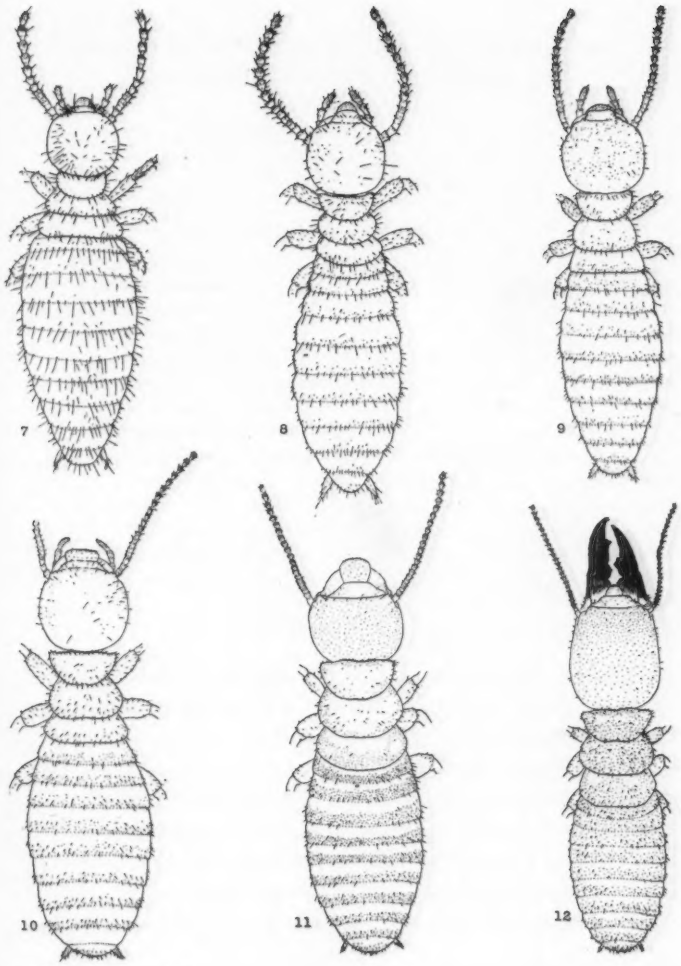
FIG. 8. Second instar; head width 1.0 mm.

FIG. 9. Third instar; head width 1.24 mm.

FIG. 10. Fourth instar; head width 1.79 mm.

FIG. 11. Fifth instar; head width 2.6 mm.

FIG. 12. Fully developed soldier; head width 2.97 mm.



H. HEATH AND B. C. WILBUR.

UNIV. OF MICH.

MATURATION PHENOMENA IN ARTIFICIAL PARTHENOGENESIS OF *ASTERIAS FORBESII.**

H. W. BEAMS.

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INTRODUCTION.[1]

Recent studies in artificial parthenogenesis of eggs that are immature when activated has resulted in several views as to the mode of maturation which may lead to normal development. Professor Tharaldsen found as a result of his previous observations of the maturation phenomena in *Asterias forbesii,* which may be artificially activated before the maturation process is completed, that this process was subject to wide variations. He proposed therefore that I make a study of the maturation phenomena in Asterias to ascertain the cytological details of the various types.

* Contribution from the Zoölogical Laboratory, Northwestern University, Evanston, Ill.

[1] I wish to acknowledge my indebtedness to Professor C. E. Tharaldsen for the use of his material and his kind assistance during the progress of this work. I also wish to express my appreciation of the helpful suggestions given by Professor A. E. Cole and Mr. G. B. Pickwell.

Material and Method.

The material on which these observations were made consisted of serial sections of *Asterias forbesii* eggs fixed in sublimate acetic (2.5 per cent. acetic acid), sectioned at 5 μ, and stained in Heidenhain's hæmatoxylin. The eggs were procured, artificially activated, and the cytological technique performed by Professor C. E. Tharaldsen during the summers of 1921–22–23, at the Marine Biological Laboratory at Woods Hole. The activating agents used consisted of the single butyric acid method as described by Lillie, and Loeb's double method.[2] The eggs had been induced to mature uniformly by a special technique similar to that described by Miss Brailey ('23). They were all activated at the same time and specimens were then fixed at intervals of one minute throughout the maturation from the breaking down of the germinal vesicle to the first cleavage. The slides were studied in chronological order.

Observations.

Normal Maturation.—The primary oöcyte of *Asterias forbesii* ranges in diameter from 100 μ, to 125 μ; and the nucleus of such eggs varies from one third to one half the diameter of the cell (Fig. 1). The nucleus is usually eccentrically located and the nucleolus is found in the nucleus on the side nearest the periphery of the egg. When activated during the breaking down of the germinal vesicle, the nucleus shows a highly chromatic alveolar network; and within this network is a very delicate achromatic (linin) reticulum. The nuclear wall begins to wrinkle and dissolve away at a point nearest the periphery of the egg. The dissolution of the nuclear membrane extends away from this point in all directions, and becomes complete at the antipode on the median side of the nucleus. The alveolar structure of the cytoplasm has itself become finer. The loss of the nuclear membrane allows a slight spread of the chromatin; so that the mass seems to thin out progressively from the center; although a quite definite demarcation between chromatin and cytoplasm always remains. At this time a small aster with its centrosome makes its appearance at a point just

[2] See Tharaldsen, "The origin and nature of cleavage centers in echinoderm eggs." Jour. Exp. Zoöl., V. 44, no. 1.

outside the spot where the nuclear wall first disappeared. The aster has apparently arisen "de novo" as described by Tennent and Hogue ('06), Scott ('06), and Tharaldsen ('26). The nucleolus fragments and apparently gives rise to chromosomes which can be seen resting free in the nuclear material (Jordan, '08; Wilson, '01). The aster enlarges for a time sending its rays well into the nuclear mass where they become attached to the chromosomes (Fig. 2). The aster together with its centrosome then divides to form the amphiaster of the first polar division (Fig. 3). The first polar spindle is formed in a position tangential to the surface of the egg. When the mitotic figure has reached its maximum size it rotates at right angles to this position, and migrates to the surface of the cell (Fig. 4). Here the chromosomes divide and move to the foci of the two centers (Fig. 5). With the extrusion and pinching off of the first polar body the inner half of the amphiaster with its central body persists for a short time in the outer region of the cytoplasm after the astral rays are lost. The central body soon fades away, leaving a deeply stained area which gradually disappears. The chromosomes migrate a short distance deeper into the cytoplasm of the egg.

On the median side of the scattered chromosomes a small centrosome and central body appears. An aster is formed (Fig. 6) which divides, and the two centers move apart, drawing the chromosomes into an equatorial position. When the asters reach an antipodal position the spindle rotates approximately 90 degrees and migrates to the surface of the egg. Division takes place and the extrusion of the second polar body follows (Fig 7.).

After the extrusion of the second polar body the inner half of the amphiaster together with its centrosome and chromosomes persists for a short time, but soon degenerates as did the first, leaving the chromosomes in a deeply stained area. These give rise to chromosomal vesicles which are drawn together to form the egg nucleus by a monaster (Fig. 8). This condition has been described by Tennent and Hogue ('06) and Tharaldsen ('26). After the vesicular monaster has performed its function it disappears, leaving its center as a darkly stained body at the periphery of the newly formed nuclear membrane. The deeply stained area soon vanishes, followed shortly after by the disappearance of the

centrosome. The egg nucleus moves through the cytoplasm to a position nearer the center of the egg (Fig. 9).

At no time in the maturation division are all the chromosomes in the same stage of division, a condition which renders it very difficult to obtain the exact chromosomal count, but never has there been observed in this material more than the haploid number after the extrusion of the two polar bodies. The extrusion of two polar bodies is the usual type of maturation in *Asterias* following artificial activation, and as Tharaldsen ('26) has shown, the only type that will give rise to normal swimming larvæ.

Eggs that Extrude One Polar Body.—In some eggs the first maturation division is completed normally as above described, and the first polar body is extruded. The polar body may be retained under the limiting membrane of the egg in a compressed position, or may be pinched off in the usual manner (Figs. 12–13). The second polar spindle forms normally, but does not rotate in its usual manner, nor migrate to the surface of the egg, but remains submerged in aposition in the immediate proximity of its origin (Fig. 10). Thus, it forms two daughter nuclei which are apparently of equal size (Fig. 11) and possess equal amounts of chromatin material. The nuclei later fuse (Figs. 12–13) to form an unusually large pro-nucleus. During the fusion of the two nuclei an aster makes its appearance near the point of fusion and sends its rays out along the sides of the nuclei (Fig. 12). It may divide to form the amphiaster of the first cleavage spindle, as seen in Fig. 14. A transverse section through the equatorial plate of the first cleavage spindle of such eggs shows clearly more than the haploid number of chromosomes (Fig. 15). In some cases these nuclei may not fuse, but develop monasters that do not divide. These eccentric monasters may complete the first cycle without division and then become secondarily united with a cytaster to form the second polar spindle. This spindle may migrate to the surface of the egg and extrude the second polar body. It is also common here too for the monaster pole of the spindle to divide, giving rise to a tripolar spindle. These tripolar figures in this material have never been observed to extrude the second polar body.

Eggs that Extrude No Polar Bodies.—Some eggs have been ob-

served in which the first polar spindle may apparently become directly the first cleavage amphiaster (Fig. 16). In this case the first polar spindle is formed deep within the cytoplasm near the center of the cell. It is very much larger than the normal, and the ensuing division results in two almost equal blastomeres. A careful search through the egg gives no indication of polar bodies having been formed. Apparently the amphiaster of this figure is the first polar spindle which has become very much enlarged. It seems to form in the usual manner, but instead of migrating to the periphery of the egg, it recedes deeper into the cytoplasm.

It is common also for the first polar amphiaster to form deep within the cytoplasm and divide to form two nuclei similar to those formed by the submerged second polar spindle. The two nuclei have been observed to fuse and give rise to the cleavage nucleus. A monaster arises at the point of contact and by division forms the amphiaster of the first cleavage spindle.

Some eggs have been found where the first polar division is carried through submerged, and gives rise to two nuclei (Fig. 17). In Fig. 18 one of the nuclei has developed asters apparently simultaneously, and is migrating to a central position of the egg. Here it will become directly the first cleavage amphiaster which may divide the egg into two equal blastomeres. I have been unable to trace the fate of the other submerged nucleus, except to observe that it comes to rest in one of the blastomeres. It seems probable, however, that it might develop an aster which would divide to form the cleavage amphiaster of the blastomere.

The two nuclei that are formed by the first submerged polar spindle may develop asters which form amphiasters and divide the two nuclei, forming four nuclei in the egg. These four nuclei have been observed to fuse and form the zygote of the egg.

Monocentric Mitosis.—When a one-aster egg is studied soon after activation, it cannot be determined at that time whether the aster will later become bipolar and give rise to an amphiaster, or will only pass through rhythmic fluctuations that never lead to division. These eccentric monasters pass through growth and recession stages which may or may not be repeated. These cycles correspond in time, roughly, with the formation of the first polar amphiaster, the second polar monaster, and amphiaster of the

second polar division. In each cycle the rythmic disappearance and reappearance of the rays and successive division of the chromosomes take place. In Fig. 19 the rays are extended and the chromosomes are scattered in an arc midway the length of the rays. The chromosomes are dividing and will later be recollected at the nucleus where the astral rays are focused. Fig. 20 shows the completion of a monocentric cycle in which the astral rays have been lost and the double number of chromosomes are resting free in the cytoplasm. I have observed eggs where the chromosome count showed they had passed through three monocentric cycles, which were comparable to the first two polar and the first cleavage divisions. In Fig. 21 the first polar division has been carried through normally and the first polar body extruded in the normal way. The second maturation aster has failed to divide and is going through monocentric cycles. The astral rays are receding and the chromosomes have been collected at the nucleus. The astral rays are soon lost to view entirely and the deeply stained zone with a central body persists for a short time; but it, too, is soon lost.

Some Unusual Types.—Many very abnormal conditions of nuclear mitosis arise in eggs that have been over-exposed to the activating agent. Fig. 22 shows an early phase of a tripolar condition in which the asters have taken a triangular position around the chromatin material. This figure is the result of a simultaneous appearance of two centers, such as described by Mead ('98) for the first polar amphiaster in *Chætopterus*. One of the centers has divided and migrated to a position where the force is at equilibrium between the three centers. The other aster has apparently divided, but the central bodies remain close together, forming a wide focus for the astral rays. In some cases the two primary polar centers divide and move apart to form a typical tetraster, as described by Boveri for dispermatic eggs. In such abnormal conditions normal development never follows which is probably due to the abnormal combinations of the chromosomes.

The primary maturation monaster has been observed to divide into two, three, or more centers, giving rise to triasters, tetraster, or multiple asters. These various abnormal astral figures may give rise to several nuclei within the egg. This type of abnormal

condition is usually found after a longer exposure to the activating agent.

Fig. 23 is an equatorial plate stage of a tripolar second maturation spindle. The first maturation division has been carried through normally and the astral rays of the distal pole are fading away; while the second polar spindle has united with a cytaster which will divide the chromosomes somewhat unequally among the three centers, thus forming three nuclei with unequal chromatin content.

Fig. 24 shows a telophase of the above tripolar condition. The chromosomes have all been collected at the centers, and are broken down. The asters are beginning to recede and the result is three separate nuclei. These nuclei may or may not fuse. In most cases they have been observed to fuse (Figs. 25–26). In such cases where fusion takes place, all trace of the astral radiations, centrosomes and centrioles, of the three centers are completely lost to view. The chromatin material that has resulted from a fusion of the three nuclei may develop an individual monaster which may never divide; or in some instances they may form an amphiaster which becomes the first cleavage spindle. If the three nuclei do not fuse but remain separate, each nucleus may develop a monaster which forms an amphiaster; this divides the chromatin material again, so that six small nuclei results within the egg (Fig. 29). In another case the two nuclei resulting from the first or second polar mitosis (Figs. 11–17) have formed asters and divided again (Fig. 27) to form four small nuclei, as seen in Fig. 28. In Fig. 28 the four nuclei are in pairs. Each of the two pairs has developed an aster at its point of contact and is fusing to form two large nuclei as in the original case.

Fig. 29 shows six nuclei with asters which are the result of a division of a triaster which forms three nuclei, on each of which an aster has developed. These asters divide, thus bringing about a submerged mitosis, forming three sets of small nuclei without division of the cytosome. These six nuclei may fuse to form three nuclei again, which may in turn fuse to form the cleavage nucleus. Here again the chromosomal count could not be made, due to the many divisions of the chromosomes.

In many eggs several asters and cytasters have been observed.

In Fig. 30 three asters have become attached to chromatin material, forming a triastral condition with two independent cytasters nearby in the cytoplasm. In all cases where cytasters are found associated with nuclear material it is through secondary union. The cytaster is usually much smaller than the nuclear aster, but may vary in size and intensity, as described by Tharaldsen ('26) in *Asterias*. Usually the larger the number of cytasters present the smaller they are in size. Cytasters never divide unless associated with nuclear material; neither do they appear till after the breaking down of the germinal vesicle.

In other cases asters are seen to appear around masses of nuclear material which has failed to vacuolate and fragment in the usual manner. In Fig. 31 a group of asters are present, each of which possesses a portion of the nuclear mass which has failed to be transformed into chromosomes.

The Effect of the Double Method of Activation on the Maturation Divisions.—These observations show that the effects of the two activating agents (butyric acid and Loeb's double method) on the maturation divisions of *Asterias forbesii* initiate similar maturation phenomena within the egg. The first activating agent may cause membrane elevation only, or membrane elevation and monaster formation together with complete maturation, including its abnormal modifications. The second activating agent may resume the activation process and carry it to completion from any point where the astral phenomena initiated by the single agent halted. Either one of the activating agents may be used without the other, or the hypertonic may be followed by the butyric, or their sequence may be reversed. Also both methods give rise to apparently the same type of abnormal phenomena, including eccentric monasters, triasters, multi-asters, and submerged polar divisions. Eggs treated by Loeb's double method, however, give rise to larger polar spindles, more distinct asters, and produce more rigorous maturation phenomena in general than eggs treated by the butyric method alone. A higher per cent of abnormalities in the maturation divisions have been observed to take place after the treatment with Loeb's double method.

It therefore appears from these observations "that from the point of view of astral phenomena, the two agents are not in dif-

ferent categories with respect to their effect on the egg, but they both produce similar results in a continuous process of activation, the second carrying forward the activation effects caused by the first, whatever that may have been."

Discussion.

Cytological studies on the maturation phenomena of artificially activated eggs of *Asterias,* show this process is subject to wide variations in the history of both the chromatic and achromatic figures. It has been shown that maturation may take place with one, two, or no polar bodies extruded. The eccentric monaster is the primary phase of both achromatic figures, which in eggs properly activated may by division give rise to normal amphiaster and lead to the extrusion of each polar body. This process is also subject to wide variation both in number and arrangement of nuclear asters and their secondary union with cytasters.

The evidence in this paper shows the normal type of maturation following artificial activation involves the extrusion of two polar bodies. The dissolution of the nuclear membrane is followed by the appearance of a minute aster in the cytoplasm just outside the remnant of the germinal vesicle at a point nearest the surface of the egg. The aster divides to form the amphiaster which extrudes the first polar body. The inner aster with its central body soon degenerates and is soon lost to view. The second polar spindle is formed by the division of a monaster that arises "de novo" on the median side of the scattered chromosomes. The second polar spindle extrudes the polar body and degenerates as did the first polar spindle. Tennent and Hogue ('06) maintain that this is the only type of maturation that will give rise to normal larvæ.

The second most common type of maturation in artificially activated eggs of *Asterias forbesii* involves the extrusion of only one polar body. The polar spindles are formed as in normal maturation, but the second polar spindle fails to migrate to the surface of the egg and extrude the polar body. Division takes place submerged and forms two nuclei with apparently normal chromatin content. The usual subsequent action of these two nuclei is to fuse and form the cleavage nucleus with the diploid

number of chromosomes. This type of maturation has been described by Buchner in *Asterias,* Lefever in *Thalassema,* Allyn in *Chætopterus,* Morris in *Cumingia,* and Brauer in natural parthenogenesis of *Artemia.* These investigators report that apparently normal larvæ may develop from this type of maturation and the embryos possess the diploid number of chromosomes. It appears that normal development might follow this type of maturation in Asterias when the chromatic and the achromatic figures appear normal. The same chance of normal chromosome combination is present as that which exists in fertilization, which is in harmony with the conclusions of Boveri that normal development is dependent on the normal combination of chromosomes. It would, therefore, seem to be quite possible that another type of normal development following artificial activation may take place in *Asterias* which has a diploid number of chromosomes present.

In few cases no polar bodies are extruded following artificial activation. The first polar spindle may become directly the first cleavage amphiaster or the first polar amphiaster may divide submerged and form two nuclei which fuse to form the cleavage nucleus. Tharaldsen states he has never observed normal development following such abnormal conditions, which is probably due to the abnormal chromosome combinations.

In some cases the maturation monasters do not divide but pass through definite cycles of activity involving the rhythmic disappearance and reappearance of the rays and successive divisions of the chromosomes without division of the aster or the cytosome. Wilson ('01) has described five successive cycles of the monaster which correspond to the two maturation divisions and the first three cleavage divisions. In eccentric monaster eggs, at the telophase of the cycle, the chromosomes collect at the focus of the rays as the astral radiations disappear and give rise to a nucleus with many chromosomes. Such eggs never cleave and activity soon halts, followed by the degeneration of the monaster.

The origin and nature of the central bodies in maturation are similar to that described by King ('06) and Tharaldsen ('26) in *Asterias.* The monaster of the first polar division appears "de novo" just outside of the remnant of the germinal vesicle in the cytoplasm. It divides to form an amphiaster which extrudes the

first polar body. Mathews ('01) claims to have been able to trace the origin of the centrosome from a small granule (which divides into two bodies before passing out of the germinal vesicle) within the nuclear membrane, and is of the opinion that these bodies migrate to the periphery of the chromatic material and become the centers of the first polar division. Careful search through the chromatin material in *Asterias* before maturation sets in shows no structure that might be comparable to that described by Mathews. If there are such structures they are indistinguishable from the other granules of the cytoplasm. In no case has it been possible to identify by the usual criteria small, dark-stained bodies that give the appearance of a centrosome in the cytoplasm of the egg. Mathews has described the rupture of the nuclear wall where the central bodies pass from the chromatin material into the cytoplasm. These experiments show no evidence of such a condition existing in *Asterias*.

In the formation of the second polar spindle the evidences in this paper agree with Tennent and Hogue ('06), and King ('06), that the amphiaster is formed by the division of a monaster that has arisen " de novo " on the median side of the scattered chromosomes. The second polar spindle rests tangential to the periphery of the egg until it has reached its normal size and the chromosomes are pulled into the equatorial plate. The spindle then rotates and migrates to the periphery of the egg and extrudes the second polar body. Mead ('98) and Jordan ('08) believe they have definitely observed the outer aster of the second polar division to appear at the old-mid-body in the position of the old equatorial plate of the first division. The second polar spindle has been definitely observed in this material to be formed by the division of the secondary monaster and comes to lie in a position tangential to the periphery of the egg. No trace of a mid-body could be found as described by Mead and Jordan after the disappearance of the first polar spindle. Therefore, these evidences show that the contentions of Mead and Jordan do not apply to this material.

Buchner has described the centrosome of the second polar spindle to persist and give rise to the first cleavage amphiaster. He contends that the inner centrosome of the second maturation

division, after the astral rays have been lost, follows the pronucleus to its position near the center of the cell. This evidence supports the views of Van Beneden and Boveri that the centrosome is a permanent cell organ handed on by division from one cell to the other. I have been unable to find in this material evidence which shows central bodies or astral rays accompanying the chromosomes to their position of rest near the center of the cell, after the second polar body has been extruded.

The evidence in this paper shows that the central bodies of the achromatic figure are not a genetically continuous structure, but arise " de novo " as shown by their appearance and disappearance with each polar division. This is further illustrated by the fact that in abnormal conditions two centers may arise simultaneously to form the centers for the first or second polar spindle. In very abnormal conditions a great many such centers may arise simultaneously; a condition which precludes the possibility of a genetic continuity between centers. Therefore, the contentions of Boveri, Buchner and Mathews of the genetical continuity of central bodies are not tenable for maturation in *Asterias*.

The achromatic figures may be distorted and further complicated by secondarily uniting with cytasters. Such secondary union with cytasters may give rise to amphiasters, triasters, or a multi-asteral condition. It is impossible for such types to give rise to normal embryos. Boveri in his experiments with dispermic eggs has long since demonstrated the qualitative difference in chromosomes and the necessity of their proper combination. It would, therefore, be apparent that equivalent and fully potent daughter nuclei will result only from regular nuclear division with two centrosomes; and that when three or more controsomes are present the single daughter nuclei will not contain the full number, or proper combination of the chromosomes, and as a rule, not qualitatively the same nuclear material. Thus, the chance for normal chromosome combination in triasters and multi-asters is far less probable than in the amphiaster condition.

These observations have shown that the single and the double method of activation initiate similar maturation phenomena in *Asterias forbesii*. The double method does not produce any condition or in any way change the mechanics of the maturation

process from that observed in the single method. Either agent of the double method may be used alone, and, if given proper exposure, will produce the same effect. The second agent therefore simply accelerates the activation initiated by the first. There may be all degrees of activation and resultant astral response by either one or both the agents employed. Therefore, Herlant's and Herbst's contentions that in the double method the first activating agent produces a monaster, and the second agent produces a cytaster which unites with the monaster to form either of the polar spindles, is untenable. This contention not only does not agree with observed conditions in *Asterias,* but is totally unlike anything occuring in the normal process; and it has been shown that, following artificial activation, maturation must proceed normally in order that normal development may result.

Conclusions.

1. Maturation in artificially activated eggs of *Asterias* may involve the extrusion of one, two, or no polar bodies, but only eggs that extrude two polar bodies apparently develop normally. It seems possible that eggs extruding one polar body might develop normally but it has not been demonstrated in this material.

2. These observations have shown that eggs artificially activated by (1) butyric alone, and (2) butyric acid followed by hypertonic sea-water, are affected similarly in their maturation processes. The second agent in the activation process seems to intensify the stimulus of the first.

3. Cytasters apparently arise " de novo."

4. The primary phase of each maturation amphiaster is a monaster which arises " de novo " and subsequently divides to form the dicentric figure.

5. The extrusion of two polar bodies, which has been shown to be the normal method of maturation under artificial activation, results in the presence of a haploid number of chromosomes within the embryo which develop normally. The normal diploid number of chromosomes can only be obtained by the method whereby the second polar body fails to be extruded, which may possibly give rise to normal embryos.

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PLATE I.

Explanation of Figures.

All drawings were made from camera lucida sketches at table level, with 43 × objective and 10 × eyepiece. Drawings reduced approximately one-half in reproduction.

FIG. 1. Primary oöcyte.

FIG. 2. First polar monaster before division.

FIG. 3. Division of the first polar monaster.

FIG. 4. First polar amphiaster before extrusion of first polar body.

FIG. 5. Late anaphase of the first polar amphiaster.

FIG. 6. Second polar monaster before division.

FIG. 7. Anaphase of the second polar spindle.

FIG. 8. Vesicular monaster.

FIG. 9. Egg after completion of maturation.

FIG. 10. Late anaphase of a submerged second polar spindle.

FIG. 11. Two submerged nuclei of the second maturation division.

FIG. 12. Start of fusion of the two submerged nuclei of the second polar division. An aster has made its appearance near the point of contact.

FIG. 13. Advanced stage of the formation of zygote by fusion of two submerged nuclei formed from division of submerged second polar spindle.

FIG. 14. Early amphiaster of first cleavage spindle in which the zygote had been formed by fusion of two submerged nuclei of the second polar division.

FIG. 15. Transverse section through the equatorial plate of first cleavage spindle of an egg in which the zygote had been formed by fusion of two submerged nuclei.

FIG. 16. First cleavage amphiaster that has been formed directly without extrusion of polar bodies.

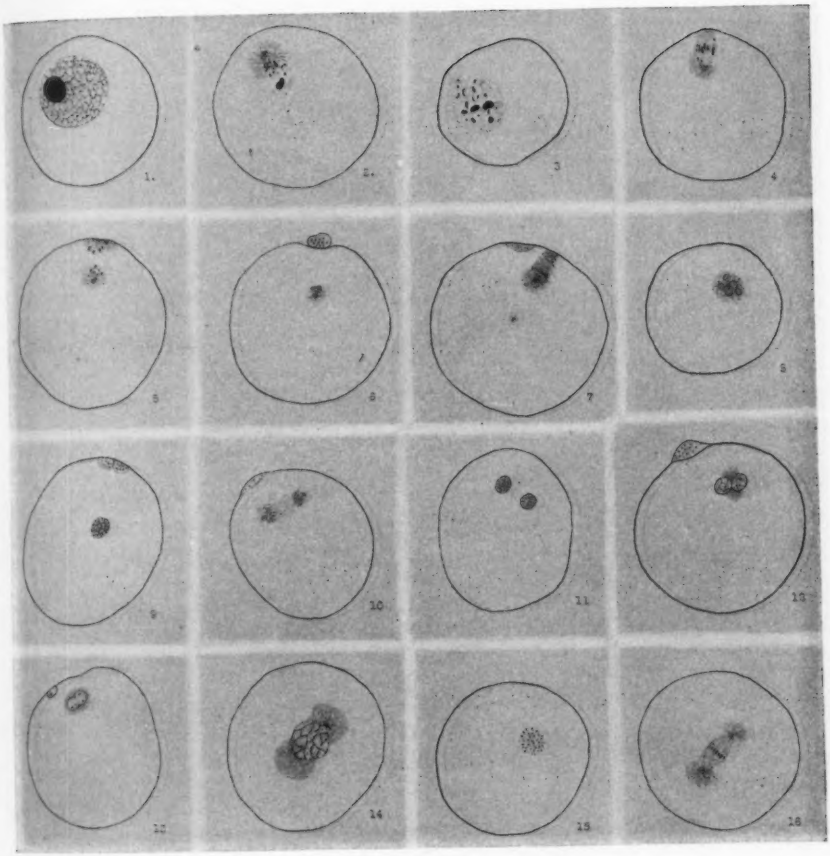

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PLATE II.

FIG. 17. Two nuclei formed by a submerged first polar spindle.

FIG. 18. Early first cleavage amphiaster formed from a submerged nucleus of the first polar spindle.

FIG. 19. Eccentric monaster first polar division.

FIG. 20. Resting stage of a second polar monaster that shows approximately 50 small chromosomes.

FIG. 21. First cleavage concentric monaster.

FIG. 22. Prophase triaster first polar division.

FIG. 23. Triaster second polar division.

FIG. 24. Late telophase triaster first polar division.

FIG. 25. Fusion of three nuclei formed by triaster of second polar division.

FIG. 26. Fusion of three nuclei that was formed from triaster of first polar division.

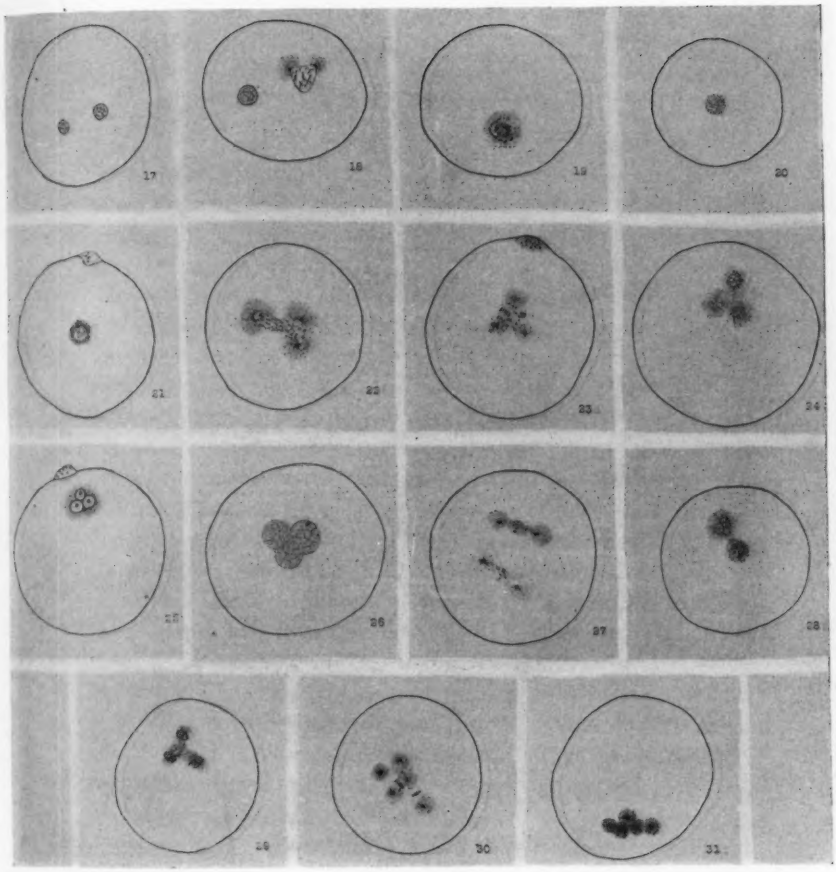
FIG. 27. Amphiasters of two submerged nuclei formed from the first or second submerged polar divisions.

FIG. 28. Four nuclei formed from division of two submerged nuclei.

FIG. 29. Six nuclei formed from division of three submerged nuclei.

FIG. 30. Triaster and cytasters.

FIG. 31. Asters formed around masses of chromatin material.



H. W. BEAMS.

THE CROSS FERTILIZATION OF ENUCLEATED *ECHINARACHNIUS* EGGS BY *ARBACIA* SPERM.

HENRY J. FRY.

WASHINGTON SQUARE COLLEGE, NEW YORK UNIVERSITY.

If a pluteus can be produced when an enucleated egg of one Echinoderm is activated by a sperm of another species having different larval characters, information may be secured concerning the respective rôles of chromatin and cytoplasm in heredity and development.

Investigations of this problem down to 1924 have been reviewed by Morgan ('24). Boveri's early experiments ('89, '95, '14) with *Sphærechinus* eggs activated by *Echinus* sperm are invalidated by uncertain enucleation, as pointed out later by Boveri himself ('18). When eggs are shaken to pieces and supposedly enucleated fragments are picked out, he found that if they are studied in section some of them are seen to contain chromatin undetectable in the living condition. Since he obtained enucleated eggs by the shaking method, the larvæ produced do not yield dependable evidence due to the possible presence of some maternal chromatin. Morgan ('96) working with the same species attempted to activate enucleated eggs of both of them with sperm of the other, but there was no development. Godlewski ('06) secured four maternal gastrulæ when enucleated *Echinus* eggs were activated with *Antedon* sperm, but his work, like Boveri's, is invalidated by the fact that the supposedly enucleated eggs, obtained by the shaking method, probably contained undetected maternal chromatin.

Taylor and Tennent ('24) satisfied the requirement of unquestioned enucleation by cutting eggs singly with a microdissection apparatus. Of forty-nine enucleated eggs of *Lytechinus* inseminated by *Tripneustes* sperm fifteen cleaved and one formed a blastula without mesenchyme. In the reverse cross only membranes were formed and there was no cleavage. They note that their investigation is not conclusive since *Tripneustes* was not in good condition.

In the present investigation[1] 689 enucleated eggs of *Echinarachnius* inseminated by *Arbacia* sperm produced forty-six blastulæ. Since the embryos died at about twelve hours, before maternal and paternal characteristics could be distinguished, a critical analysis concerning nuclear and cytoplasmic inheritance was not secured. Certain observations were made, however, a report of which may be of value to future investigations.

Dry eggs freshly obtained from the gonopores (Just, '19) do not give a higher percentage of activation, after enucleation, than eggs freshly obtained from the surface of the ovaries.

Eggs were enucleated one at a time by a glass needle held in the hand (Fry, '24). The cutting is done on an ordinary microscope slide placed on the stage of a dissecting microscope. This permits more rapid enucleation than can be accomplished with the machine as about twenty eggs can be enucleated in ten minutes. Hence it is possible to experiment with relatively large numbers of enucleated eggs rather than with several at a time, thus increasing the chance of securing a significant result. That the method gives sure and certain enucleation was shown in an earlier study (Fry, '25).

Enucleated eggs of *Echinarachnius* are viable only when fragmented at temperatures between 15° C. and 20° C. If the cutting is performed at temperatures either below 15° C. or above 20° C., there is practically no activation. The microscope is placed in a box having a glass top through which the oculars project. Openings in the front permit the entrance of the hands. The temperature is roughly controlled by ice containers. Since the air within the box is thoroughly moist, this reduces evaporation from the drop of water on the slide that contains the eggs and fragments. Thus changes in osmotic pressure are reduced to a minimum.

No effort was made to locate the micropyle by the use of inks and the eggs were cut by chance in any plane. Therefore, no data were secured as to the relation between cutting in different planes and the resultant effects upon the development of enucleated fragments.

[1] The experiments were carried on at the Marine Biological Laboratory at Woods Hole, Massachusetts, during June and July 1926.

The majority of the enucleated eggs were about three fourths the size of a whole one. None were less than one half size and many were nine tenths size or even larger since every effort was made to remove the nucleus with a minimum amount of cytoplasm. Within these size limits, from one half to nine tenths, there is no correlation between the size and the capacity to respond to activation by *Arbacia* sperm. This indicates that failure to secure development in such experiments is not due to the size of the fragments if they be at least half the size of a whole egg. The lowest size limit capable of activation was not ascertained.

Just ('19) found that the best method of activating *Echinarachnius* eggs with *Arbacia* sperm is by the use of a heavy sperm suspension. In the present experiment with enucleated eggs various dilutions of sperm suspension yielded about the same percentages of activation, so long as the suspension was at least three drops of seminal fluid to 50 cc. of sea water. Heavier suspensions do not modify the amount of development.

Whether the enucleated eggs are activated a few minutes after cutting, or as late as an hour afterwards, the percentage of development is the same. A period of recovery from so-called "shock" is not required.

The 689 enucleated eggs yielded forty six blastulæ, giving about six per cent. development. Whole *Echinarachnius* eggs when similarly activated with *Arbacia* sperm yield an average of eighteen per cent. development (Just, '19). This difference in percentage of development is probably a result of the general maltreatment of enucleation.

It would be expected that there might be a considerable amount of irregular development among enucleated eggs caused by polyspermy. In most cases, however, either the enucleated eggs cleave regularly or they do not cleave at all, and there is little irregular cleavage. Although polyspermy and consequent irregular cleavage occur in fragments obtained by shaking eggs to pieces (Morgan, '96), polyspermy, as judged by irregular cleavage, is rare in fragments obtained by cutting with a glass needle. The shaking method is probably quite harmful to the egg and wrecks its mechanism for controlling polyspermy, whereas the glass hair leaves it in a more normal condition.

Embryos were reared in 5 cc. round-bottom salt cellars under the conditions described by Tennent ('10). Great care was taken to use sterilized sea water, to change it several times a day, to prevent evaporation, and to keep it at the temperature of the running sea water in the aquaria.

The blastulæ appeared quite regular but were more frail and delicate than normal ones. Most of them died at about twelve hours. Mesenchyme cells were not observed. Five blastulæ lived about two days; they were top swimmers, but became opaque and underwent no invagination.

In his final work Boveri ('18) came to the conclusion that completely enucleated eggs of *Sphærechinus*, as identified by the small size of the haploid nuclei, when activated by *Echinus* sperm rarely produce a gastrula and seldom, if ever, produce a pluteus. Taylor and Tennent ('24) secured but one blastula, and it did not gastrulate, in the cross between enucleated *Lytechnius* eggs and *Tripneustes* sperm. The present experiment yields a similar result since the forty-six blastulæ failed to gastrulate.

Enucleated eggs of *Echinarachnius* when activated by *Arbacia* sperm produce blastulæ that do not gastrulate whereas whole eggs similarly activated produce gastrulæ and plutei. This difference in development between enucleated eggs and whole ones when cross fertilized might be attributed to the general harmful effects of enucleation involving general maltreatment and the loss of some cytoplasm. This is probably not the cause, however, since the enucleated eggs are in a sufficiently normal condition to be activated by the sperm, to effect cleavage, and to form blastulæ. The probable explanation of the difference in development lies in the fact that the whole eggs contain a haploid set of their own chromosomes that normally function in that cytoplasm, in addition to the other set of another species introduced by the sperm, whereas the enucleated eggs contain only the chromosomes of another species functioning in an alien cytoplasm. The experiment demands that the two species used shall not be too closely related, for they must be sufficiently separated to have different pluteus characteristics in order that the respective rôles of maternal cytoplasm and paternal chromatin

may be analyzed by those very differences. It is probably a significant comparison that *if an Echinarachnius egg is activated by the sperm of a somewhat distantly related species, development goes through to pluteus formation if the maternal chromosomes are present, but does not go beyond the blastula stage if the maternal chromosomes are absent.* The exact meaning of this involves an explanation of the interaction of the various factors concerned at various stages of development in whole eggs and enucleated ones, *i.e.*, the maternal cytoplasm into which was released maternal nuclear substances at maturation; the later effects of maternal genes; and the later effects of genes of another species. It is such an analysis of these factors that this type of experiment is designed to effect, but the failure of the embryos from enucleated eggs to develop beyond a blastula prevents an analysis of the problem. Whether or not a gastrula and pluteus can develop from an enucleated egg of one species when activated by the sperm of another species, that is sufficiently distant in relationship to have different larval characters, awaits the results of further experiments on various forms.

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A PRELIMINARY REPORT ON THE STRUCTURAL ELEMENTS OF THE CYTOPLASM IN PLANT CELLS.

ROBERT H. BOWEN,

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY.

ONE PLATE.

In several recent papers (Bowen, '26*a*, *b*, *c*) I have reported briefly on some aspects of cytoplasmic structure in plant cells, as revealed in the first stages of an extensive study of the problem as a whole. This investigation has now been pushed considerably farther and much more complete results are in hand. Errors in my earlier reports, both of omission and commission, have now become evident, and I propose in this paper to restate the major results by way of correcting and extending my previous accounts, pending the completion of more extended papers, now in preparation.

MATERIAL.

My study thus far embraces material from each of the three higher plant groups, Bryophyta, Pteridophyta and Spermatophyta, as follows:

Bryophyta—male heads (including developing sperm cells, antheridial epithelium, and cells of the paraphyses and leaves) of *Polytrichum juniperinum*, *piliferum* and *commune*.

Pteridophyta—growing-point and root-tip of *Equisetum arvense*.

Spermatophyta—young shoots of barley and root-tips of *Vicia*, *Pisum*, *Ricinus*, *Hyacinthus*, barley, pumpkin and kidney bean.

Some of this material has received as yet only preliminary study, but so far as the results go, there appears to be a surprising uniformity in plant cells of all kinds as regards the general morphology of the formed elements of their cytoplasm.

METHODS.

The problem of *how* to study plant cells is one which seems to have received astonishingly little attention. The most ex-

tensive work in recent years on plant cytoplasm is undoubtedly that of Guilliermond, who arrives at the conclusion that the fixatives involving osmic acid, so long used by zoölogists, give very erratic and misleading results on plant tissue and are therefore in general to be avoided. He himself and his students employ a method which is essentially one of Regaud's formulæ, and which in Guilliermond's laboratory seems to have yielded excellent results. I have given this method extensive trial on root-tips with most unhappy results, and have finally discarded it completely as unusable in my hands. The details of the method have never been published very fully, and presumably I have made some unavoidable technical errors which had fatal results. In place of this method I have concentrated on the methods long used by zoölogists in the study of animal cytoplasm, and can not agree with Guilliermond's conclusion as to their lack of value for the study of plant cells. On the contrary, I find that with proper experience and controls these methods yield results on plant cells of a value in every way equal to those obtained in animals, and in some respects superior to Guilliermond's method as successfully employed by himself. Quite a number of different methods have been tried, but the following have proved most generally useful:

Benda—using Mottier's ('18) modification of the fixative, and continuing as for the Benda method with animal tissues. This method stains the plastidome more or less specifically in meristem cells, and is very useful for separating it from other components.

Champy-Kull—following the directions exactly as given for animal tissue. This method stains the pseudochondriome (see beyond) more or less specifically and in some cases will separate it completely from all other components of the cytoplasm.

Kolatchev and *Weigl*—following the directions generally given for animal tissues, but with some slight modifications suggested by experience. These methods were first employed by me for an extensive study of plant cells, the results having been briefly published nearly a year ago (Bowen, '26*a*). Meanwhile Guilliermond ('26) had been independently working with the Kolatchev and other osmic methods and his results, published shortly after

my report, corroborate my findings in a general way, but offer some very remarkable differences in detail. My work with these methods has shown that they will impregnate practically everything in a plant cell except chromosomes (and probably central bodies when they occur). The classes of materials thus demonstrated may be tabulated as follows:

1. Osmiophilic platelets,
2. Plastidome,
3. Pseudochondriome,
4. Vacuome,
5. Nucleus—not including the nucleolus as a rule,
6. Intra-nucleolar bodies,
7. Spindle fibers, cytoplasmic network and cell plate,
8. Intra-vacuolar masses.

The outcome of any particular trial can not in general be foretold, and these osmic methods can only be interpreted with accuracy after extensive experience—and not always then. In spite of the obvious disadvantages arising from their excessive lack of specificity, these methods nevertheless yield results unequalled by any other technique, and can not fail to be of extraordinary interest and value to anyone engaged in the study of plant cells.

The methods here outlined together with others will be given in another paper the detailed consideration which space does not now permit.

Results on Root-tips.

The Osmiophilic Platelets.—Following osmic impregnation, the most generally demonstrated components of the cell are the osmiophilic platelets. I have given them this name merely as a convenient descriptive term, pending the final adjudication of their status. They have the shape of plates or discs, the periphery of which is intensely blackened by osmic acid (Figs. 1 and 8). The central part may be quite clear or may be slightly blackened or browned in varying degree. Just what the exact morphology of these bodies may be is not known, but the impression one gets is that of a plaque with a peripheral rim of specifically different composition. When seen in face view, the

blackened rim appears as a ring, while in profile it appears as a rod, ellipses of varying size being apparently seen at intermediate angles of vision. These platelets have now been demonstrated with the greatest clearness in practically all the tissues mentioned in this paper, and throughout the three higher plant groups they appear to be a constant and characteristic part of the cytoplasm. They are usually very numerous, sometimes running into the hundreds, as in the large cells of the barley root-tip. In general they are rather small (Fig. 1) but they tend to be larger in the root-cap cells of spermatophytes, and less numerous and more conspicuous in the androgonia of *Polytrichum* (Fig. 8). They show no peculiarities of distribution in either the resting or dividing cell, except possibly a tendency to heap up along the cell plate in the telophase of division. They seem to multiply by a process of fragmentation, but nothing very definite can be said on this point. Thus far they do not appear to have any function capable of expression in terms of their visible morphology, except in the moss androcyte.

The most extraordinary thing about these platelets is that they are so readily demonstrable by the methods used for the animal Golgi apparatus, but, with rare exceptions of a special nature, can not be demonstrated by any of the usual staining methods. It appears probable that these bodies as a permanent and generalized component of plant cytoplasm have never been described before, indeed may very probably never have been seen by botanists. It is an extraordinary fact that Guilliermond ('26) does not mention these platelets in his recent paper, and the only explanation I can offer is that they have possibly been mistaken for one of the other and well-known cytoplasmic constituents. He has, in an earlier paper ('24), claimed that plant cells in general contain lipoidal granules which he calls the "granulations lipoides." These are granules, not platelets, and they are easily demonstrated by osmic methods which do not suffice for the osmiophilic platelets. I am, therefore, of the opinion that these fatty granules of Guilliermond represent exactly what he calls them, and that they have nothing to do with the osmiophilic platelets here described. The lipoidal granulations would appear to correspond roughly to the fatty

granules or droplets occurring in many animal cells, and they have in all probability only secondary interest for purposes of a generalized understanding of cytoplasmic structure. In my first two reports ('26*a*, *b*) I called these platelets the "spherosomes" after the terminology of the Dangeards, and at first suggested their homology with the "inactive chondriome" of Guilliermond. Extended study of other classes of formed bodies in the plant cell has, however, convinced me that such an identification is impossible. These platelets are something new, and certainly have nothing to do with Guilliermond's chondriosomal granules, as will appear more clearly in a succeeding section.

There is, finally, nothing about these platelets in root-tip cells to furnish a critical demonstration of their status in the plant cell. Their regular response to the Golgi apparatus methods and their striking similarity to the scattered Golgi bodies of many animal cells, suggests their homology with the animal Golgi apparatus. Indeed, as will be indicated later, this is probably more than a mere matter of coincidence, but for the moment I wish merely to emphasize the lack of any conclusive evidence as to their nature so far as such evidence might be furnished by observations on root-tips.

The Plastidome.—This comprises a group of formed bodies which occur generally in plant cells, appearing in older meristem and more differentiated plant cells as centers for starch production, deposit or storage—the familiar plastids of various kinds. In the earliest meristem, the forerunners of the plastids are already present and may be called, in general, proplastids. I have found thus far no evidence whatever in root-tips that the plastidome ever has any relation to other cellular components, and everything indicates that the proplastids constitute an independent group of materials whose sole function is the production of plastids in the more differentiated cells of the plant. In root-tip cells the proplastids possess in general an elongate, thread or rod-like shape (Figs. 2 and 3). They may be blackened by osmic acid, especially in *Vicia*, but are probably best demonstrated in Benda preparations, occasionally, as in the hyacinth, with acid fuchsin. Every step in the gradual dep-

osition of starch in the plastid primordia has now been traced, especially in cells of the root-cap and periblem, and their transformation into typical plastids is undoubted. My results thus confirm the morphological results obtained by Guilliermond, who has particularly emphasized as characteristic the generally elongate shape of the proplastids in all sorts of meristems.

The plastidome is characterized further by its general tendency to be oriented in a specific way with respect to the spindle of a dividing cell. The facts have been brought out particularly by Nassonov ('18), and my results corroborate his account, and push the matter somewhat further. In Fig. 2, an especially fine prophase example of the orientation of the proplastids with respect to the future spindle is shown. It now seems more and more probable that this behavior of the plastidome in cell division is a very common phenomenon in plant cell-division, and has been long overlooked simply because of the technical difficulties which so often bring disaster at the particularly important moments of division.

Guilliermond has claimed that the plastidome is a specially differentiated part of the chondriome, characteristic of plant cells. My observations on root-tip cells do not offer any evidence in support of such an identification. Certainly these bodies offer distinct differences in staining capacity as compared to any other cellular elements in plants, and it does not seem possible to settle their status on the basis of their behavior in root-tips.

The Pseudochondriome.—This comprises a group of bodies to which Guilliermond has given the name of "inactive chondriome," in contrast to his "active chondriome" or plastidome. There are in fact several good reasons for believing that these bodies are equivalent to the chondriosomes of animal cells, but pending more complete evidence I have chosen to use the term pseudochondriome as descriptive of their similarity to chondriosomes, but without committing myself for the present to any definite view of their real nature. In my first reports these bodies were confused with the osmiophilic platelets, because of certain morphological similarities when representatives of the two classes are viewed in the proper plane. Indeed, several people

upon seeing preparations of the osmiophilic platelets and of the pseudochondriome have asked upon what basis any separation can be made. It is true that the one class of bodies is composed of (flat) discs, the other of spherical granules, but it was thought that possibly the morphological differences were due mainly to the different technical treatment. The first suspicion in my own mind of the distinction between these two components came from a study of the moss androgonia, and I eventually spent several months during the past fall trying to get critical evidence on this point. This evidence I believe is now conclusive, and the pseudochondriome and osmiophilic platelets must be considered as entirely distinct kinds of bodies.

The pseudochondriome consists of a larger or smaller number of granules scattered throughout the cell and showing no important relations to the spindle in cell division (in root-tips at least). These bodies rarely blacken with osmic acid, an exception being the hyacinth, where they are finely demonstrated as jet black granules on a clear background (Fig. 3). They are occasionally stained by Benda's method or Fe-hematoxylin, but by far the best method is that of Kull. In good Kull preparations (Fig. 4) each granule shows a typically vesicular structure, with a brightly stained periphery and a light center—a composition quite common in the true chondriosomes of molluscan and lepidopteran sperm cells, for example. In older cells these granules often show signs of elongation, etc., but their typical form in the earliest meristem is that of vesicular granules. My results accordingly agree in a general way with Guilliermond's findings, and the facts also indicate that his interpretation of these bodies as chondriosomes is also correct. But it must again be emphasized that there is nothing in root-tip cells themselves upon which to base such a decisive conclusion as to their homologies with the animal chondriome.

Finally as to the distinction which I have drawn between these bodies and the osmiophilic platelets, three lines of supporting evidence combine, it seems to me, to render it secure. In the first place, there are the obvious differences in morphology. The pseudochondriosomes are spherical, and thus, while presenting the appearance after proper staining of rings, these

optical sections are always rings, while in case of the osmiophilic platelets, the ring appearance holds from only one point of view, the plates appearing as rods or ellipses when seen on edge or obliquely. In the second place, in the hyacinth the plastidome, pseudochondriome and osmiophilic platelets can all be demonstrated in the same cell with osmic acid, and thus the distinction between all three classes can be demonstrated (Fig. 3). Finally, after blackening the osmiophilic platelets, it is sometimes possible to counterstain with acid fuchsin, thus demonstrating the platelets in black and the pseudochondriosomes in red in the same cell.

The Vacuome.—In many kinds of cells I have found that the vacuome can be impregnated with osmic acid, and thus permanent preparations of great brilliance can be secured. Such preparations have obvious advantages over the vital-staining methods usually employed heretofore. Since the fact that various methods designed for the demonstration of the animal Golgi apparatus have been used with some success for demonstrating the vacuome in plants, and since at some stages the plant vacuome may assume net-like appearances, it has been claimed by Guilliermond and others that the vacuome represents the Golgi apparatus in animal cells. Quite apart from the ultimate merits of such a claim, I wish to draw attention to certain features presented by the vacuome after osmic treatment, which at least suggest that the response of the vacuome to this technical treatment may have little or nothing in common with the blackening of true Golgi material.

There is, in the first place, a distinct difference between the action of the Weigl and of the Kolatchev methods. One may work very much better than the other on a particular kind of cell. Furthermore, the vacuome of some kinds of cells responds regularly, of others irregularly, and of still others not at all, to the same osmic treatment. In addition the type of response differs. Sometimes the vacuoles as a whole (Figs. 5 and 7) are intensely and evenly blackened; in other cases only more or less well-marked traceries of black or gray mark the vacuoles (Fig. 6), these traceries running perhaps through the substance of the vacuole as well as over its surface. Thus the suspicion

is aroused that the reaction to osmic acid is largely a response of the contents of the vacuole to osmic acid, the contents responding positively or negatively according to its composition. Thus some root-tips will respond, while in other plants the results are negative or very indifferent. This should certainly not be the case if the vacuome as a whole represents the Golgi apparatus, and the conclusion certainly seems justified, that the claims so far made are not in agreement with the known behavior of osmic acid toward the animal Golgi apparatus. In a preceding report (Bowen, '26*b*) I inclined to the belief that the vacuolar primordia had lipoidal affinities, possibly in the sense that the vacuolar walls were lipoidal in nature. Such a view still seems to me a possible one, and it would certainly make easier an explanation of the results as a whole—but whether or not such an enveloping lipoid membrane occurs, can not now be finally settled. Should such a membrane occur, it might be possible to relate it to the Golgi apparatus, but as to the contents of the vacuole, no relation with the animal Golgi apparatus itself seems possible.

The most complete results on the morphology of the vacuome have been obtained on the root-tips of *Vicia* and *Ricinus*, particularly the former. In the earliest meristem cells the cytoplasm is crowded with many small, more or less spherical vacuoles or vacuole primordia (compare Fig. 5, an older stage in *Ricinus*). In *Vicia* these vacuole primordia undergo rather different histories in plerome and periblem. In the plerome, the small vacuoles of the earliest stages gradually merge together (Fig. 6), growing larger meanwhile, until the large vacuole characteristic of plant cells emerges. All phases in the fusion can be impregnated with unusual clarity in the periblem of *Ricinus*. In the periblem of *Vicia* a remarkable network is developed by transformation and fusion of the vacuole primordia (Fig. 7), later passing on into the large single-vacuole condition. In cell division (Fig. 7, left-hand cell, prophase), these networks are frequently more or less fragmented, presenting a very characteristic appearance. It is claimed by some who have seen my preparations that such bizarre networks can not possibly be normal, but the results of the Dangeards and particularly of

Pensa ('13) on living cells indicate that these osmic impregnations are really remarkable demonstrations of the actual condition of the vacuoles. This is borne out by the fact that such networks in the periblem are characteristic only of *Vicia* (among the forms so far studied by me).

My results on the vacuoles thus bear out the recent extended researches of the Dangeards and of Guilliermond, and go far toward establishing the vacuome as an independent and permanent part of the plant cell system.

Other Cell Structures.—Of the other constituents mentioned in an earlier section as being blackened by osmic acid, none throw any light upon the general problem of cytoplasmic components, and they will not therefore receive further mention here. My results on the blackening of the spindle fibers corroborate, in the main, those of Nassonov ('18), to whose findings I have added the interesting fact that the cell plate can be blackened at all stages of its formation.

Antheridia of Polytrichum.

The results of my study of root-tips are now fairly complete, and they lead unmistakably to the conclusion that the cytoplasm of plant cells contains four classes of formed elements, which are apparently distinct and independent, and probably self-perpetuating under normal circumstances. In animal cells there are only two such generalized classes of formed bodies—chondriome and Golgi apparatus—at least so far as known at present. It is thus impossible to draw any off-hand conclusions as to what components in the plant cell are equivalent to those of the animal cell. Furthermore, the study of meristem cells in root-tips gives no conclusive answer to the problem of homologies. As a means of getting around this unhappy impasse, I suggested in a previous note (Bowen, '26*b*) the possibility of finding processes in the formation of plant sperms which would be comparable to those now known to occur universally in differentiating animal sperms. I have already reported briefly on the probability of achieving success in this direction. Further study, particularly of more successful osmic impregnation preparations, has, however, clearly indicated one or two errors

in my first account. These I wish now to correct; leaving several further points undecided pending additional study of the material as a whole. I have examined thus far the antheridia of three species of *Polytrichum—juniperinum, piliferum* and *commune*. While some minor differences doubtless occur, for practical purposes these three species may be considered as a unit basis for description.

In the androgonia and androcytes the vacuome has not yet been identified, and it is uncertain whether any occurs. The three other cytoplasmic components occur in clearly developed form. The osmiophilic platelets (Fig. 8) are similar to those in the root-tips. They tend to become smaller as the size of the multiplying androgonia diminishes, and are distributed apparently intact to the daughter cells of each division, and without any obvious orientation with respect to the spindle. They arrive in the androcytes as scattered bodies of the same shape and staining capacity as in the androgonia. The pseudochondriome is represented in early androgonia by scattered vesicular granules (Fig. 10), exactly similar to those in root-tips. In later generations of androgonia they frequently undergo changes in shape, becoming rod- or thread-like. They seem to arrive in this form in the androcyte, although on this point I am still in some uncertainty. In cell division they show relations to the spindle axis of several types which can not here be elaborated upon. The plastidome occurs in the earlier androgonia in the form of an extensive lamella or plate (Fig. 10), the polar plates of Allen ('17). In later generations these become more compact and are molded into definite rings, of which one occurs in each cell. Fig. 9 shows an antheridial tip with the epithelium enclosing the central mass of androgonia. The plastids in the epithelium and the polar plates or rings are clearly blackened by osmic acid, yielding a remarkably clear preparation. A study of young antheridia in all stages of formation indicates pretty clearly that these polar plates are directly descended from the plastids of the cells which formed the primordia of each antheridium. In the developing male cells their identity is retained but their function as starch-producing plastids is apparently suspended. They have a striking point in common

with the plastidome of root-tip cells, viz., their orientation at the spindle poles during cell division, as observed by Allen. With the spindle itself, however, I can not find that they have anything to do. I have followed all stages in the division of rings like those of Fig. 9, preparatory to cell division, but I have not been able as yet to verify Allen's account of their fragmentation in the older generations of androgonia. The mode of their distribution to, or their morphology in, the androcytes has not yet been certainly made out. In general it may be said that the plastidome of moss androgonia shows an essential similarity to that of the ordinary meristem cells of spermatophyte root-tips.

It remains now to follow the history of these various components in the androcytes as they gradually transform into sperm cells. The history of the osmiophilic platelets (called spherosomes in an earlier report (Bowen, '26*b*)) will first be indicated. In the early androcytes, the scattered osmiophilic platelets gather gradually into an irregular, elongated heap lying along one side of the nucleus. It was my first belief, borne out by hematoxylin preparations, that this was the limosphere of authors, gradually forming by fusion of the platelets. It is clear now that the whole course of events is more complicated, and that the limosphere is by no means so simple a structure as has been supposed. Actually the osmiophilic platelets are thus assembled in connection with the formation of a new structure to which it will hereafter be convenient to limit the term "limosphere," regardless of the fact that as originally used by Wilson ('11) it probably involved the platelets proper to some extent at least. The limosphere appears first amid the platelets as an elongate body, often duplex in a later stage, but finally rounding up to form a conspicuous clear sphere within which is a large darkly staining granule (Figs. 11 to 14). This is the typical limosphere of Allen's account, its earlier stages having been overlooked for the most part by previous workers. During the later stages in its development, the osmiophilic platelets show a less intimate relation to the limosphere (compare Figs. 11 and 14), and subsequently the relation becomes very loose, the platelets being even more

scattered and more independent of the limosphere or its remains. The limosphere now begins to elongate, its staining capacity (with hematoxylin or acid fuchsin) rapidly changes, and a portion of its substance is gradually separated off and deposited along the blepharoplast at the anterior end of the sperm (Figs. 17 and 18). The remnant of the limosphere (Fig. 18) takes no further part in the development of the sperm, although it undergoes further characteristic changes in morphology and staining capacity. The part deposited at the sperm tip is the so-called apical body. It begins very soon to elongate (Fig. 19), and by means of acid fuchsin I have been able to follow its later history and to show that it probably stretches out along the elongating nucleus, forming thus a rod-like "acrosome" comparable to that in some of the Pentatomidæ.

The interpretation of these phenomena seems fairly clear. The relation of the osmiophilic platelets to the developing limosphere suggests in every way the formation of the acrosomal material in those animals in which the Golgi bodies do not fuse to form a single, permanent mass—the acroblast. The structure of the limosphere is remarkably similar to that of many secretory granules and of the acrosomal vesicle and granule of animal sperms. The ultimate history of the apical body portion in the moss sperm is again exactly comparable to that of the acrosome in animals. The unexpected difference presented by the division of the limosphere does not, so far as I know, ever occur in animals. This unusual behavior together with the staining of the limosphere at this period (Figs. 17 and 18) led to my erroneous conclusion that the remnant of the limosphere was equivalent to the Golgi remnant in animals. It is clear, however, that the essential homologies which I first pointed out (Bowen, '26*b*) between the formation of the apical body and the osmiophilic platelets still hold. My conclusion that the osmiophilic platelets produce the acrosomal material in the moss sperm and that they are therefore equivalent to the animal Golgi apparatus can, therefore, now be reaffirmed. All the evidence available from sources of every kind bears out my contention that the osmiophilic platelets represent such part of the Golgi apparatus of plant cells as can now be certainly identified. It would be extra-

ordinarily interesting to know whether the vacuole primordia, more particularly their enclosing membranes, play any joint rôle in the formation of the limosphere, and might thus be brought into line with the Golgi apparatus. But thus far there is not the slightest evidence of such a coöperation, indeed at present no vacuome has been demonstrated in the androcytes, and at the moment I am inclined to the opinion that no such coöperation occurs.

The particular mistake of my earlier account, which I wish now to make clear, has to do with an error in interpreting a stage like Fig. 14. It was my belief, in the absence of an extensive series of successful Golgi preparations, that the limosphere in early androcytes was an early stage in the history of the chondriome. The blackened granule and its enclosing vacuole gave to it an appearance exactly like that of the nebenkern in an insect sperm. I believed that the limosphere proper appeared only at a later stage. The whole picture (Fig. 14) is so strikingly like an insect spermatid that the error was not suspected by myself or by others who were familiar with osmic acid preparations. As a matter of fact, the moss androcyte does possess a remarkable similarity to a generalized animal spermatid, though not completely in the manner I suggested at first.

This clears the ground for a final disposition of the plastidome and pseudochondriome in the developing sperm. As regards the plastidome I have been as yet unable to follow its history, and its identification at any stage in the androcytes is still uncertain. It is possible that it degenerates, but it seems more probable that it either remains distinct and is disposed in some characteristic place in the sperm, or merges inextricably with the pseudochondriome. In the first case it would be practically necessary to consider the plastidome as an independent cytoplasmic component equal in rank with the chondriome and Golgi apparatus. In the second case the conclusion would seem justified that the plastidome is merely a specially differentiated portion of the pseudochondriome, as has long been contended by Guilliermond. Confusion between these two components in the spermatid led me to identify the plastidome with the pseudochondriome in my earlier report, and to draw the conclusion

that the plastidome was therefore homologous with the animal chondriome. This inference now appears to have been based on inadequate evidence, and while it may be correct I prefer for the present to hold the whole matter of plastid homologies in abeyance pending my more extended study of moss material.

The pseudochondriome seems to occur in the earlier androcytes as a tangled mass of threads more or less encircling the nucleus and to some extent the developing limosphere. Eventually this material seems to spread out over the nucleus, and stretches out along the length of the sperm as the nucleus itself elongates. This general history and the ultimate arrangement of the pseudochondriome in the sperm lead me to believe that this material represents the chondriome of animal cells. That, indeed, the superficial similarities between an animal chondriosome and a plant pseudochondriosome are real, the pseudochondriome being in fact the chondriome of the plant cell, as Mottier ('18) for example has held, and also Guilliermond ('24) in the modified sense indicated above.

One further cytoplasmic component of a generalized nature remains to be considered—the central apparatus, central bodies, or centrioles, often called blepharoplasts by botanists. In general, plant cells are characterized by the absence of central bodies, and when something similar to them does occur, there has been much controversy as to whether such structures can properly be homologized with the central bodies of animal cells. Sperm-forming cells offer a remarkable opportunity for checking up on this matter, for the reason that the relation of the tail filament to the central apparatus in the sperm is beyond dispute. If in plant sperms a centriole-like body should be related to vibratile filaments, the conclusion that this body was a true centriole would appear unavoidable. According to Allen ('17) *Polytrichum* androcytes possess a small "blepharoplast" which eventually draws out into a rod-like structure applied to the elongated nucleus. The two free filaments characteristic of moss sperms appear to be inserted toward the anterior end of this rod, but Allen was able to follow their growth only in a fragmentary way. They seem suddenly to acquire a decided staining capacity in their completed condition. According to Wilson ('11) on the

other hand, the moss androcytes possess a small blepharoplast which remains morphologically distinct from a filament (the bulk of Allen's blepharoplast) that grows from it posteriorly. My studies clearly support the latter description. At an early stage (Fig. 15) there is a small, definite blepharoplast or central body to which is attached a delicate filament. Subsequently (Fig. 16), the central body becomes somewhat larger and more conspicuous (as is common also in animal spermatids), and the filament grows rapidly back over the nucleus. When the nucleus begins to elongate, its anterior tip is attached to the posterior end of the centriole (Fig. 18), a relation which probably is maintained in the completed sperm. But the extraordinary thing is that the filament, at first apparently single, soon becomes clearly multiple. Benda preparations, which stain the filament very sharply, show at least two filaments which separate sufficiently at chance places to be clearly demonstrated. There are sometimes indications of a third filament, though this seems at present to be doubtful. It is my suggestion that these two filaments are the well-known free filaments of the mature sperm. After the sperm is well along in its differentiation they probably break loose from their association with the body of the sperm, and thus Allen's failure to follow them clearly in their growth would be logically accounted for. I believe that the observations here recorded leave no doubt that the body to which the filaments are attached is a true centriole comparable in every way to the central bodies of animal cells.

This brief outline of sperm formation in mosses clears up many points which long have been a puzzle to cytologists. It leaves the fate and possible homologies of the plastidome in an unsettled condition, but I have hopes that by the time my study is ended this gap will have been closed at least in part. The results here given in outline will be reported in detail in a series of papers of which the first will soon be ready for publication.

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EXPLANATION OF PLATE I.

Figures 1, 2, 4, 6 and 7 are from root-tips of *Vicia faba;* Fig. 3 from *Hyacinthus;* Fig. 5 from *Ricinus;* Fig. 9 is from *Polytrichum juniperinum;* Figs. 8, 10, 11 and 13 are from *P. commune;* Figs. 12 and 14 to 19 are from *P. piliferum.* Figs. 1 to 7 and 9 are reproduced at a magnification of approximately 1125 ×; Figs. 8 and 10 to 19 at 2475 ×.

FIG. 1. Osmiophilic platelets. Kolatchev.

FIG. 2. Plastidome in an early prophase. Benda.

FIG. 3. Osmiophilic platelets, plastidome and pseudochondriome. Kolatchev.

FIG. 4. Pseudochondriome. Champy-Kull.

FIG. 5. Vacuome. Weigl.

FIG. 6. Osmiophilic platelets, plastidome and vacuome. Kolatchev.

FIG. 7. Vacuome and osmiophilic platelets. Weigl.

FIG. 8. Early androgonia. Kolatchev.

FIG. 9. Tip of an antheridium with androgonia in a middle phase of multiplication. Weigl.

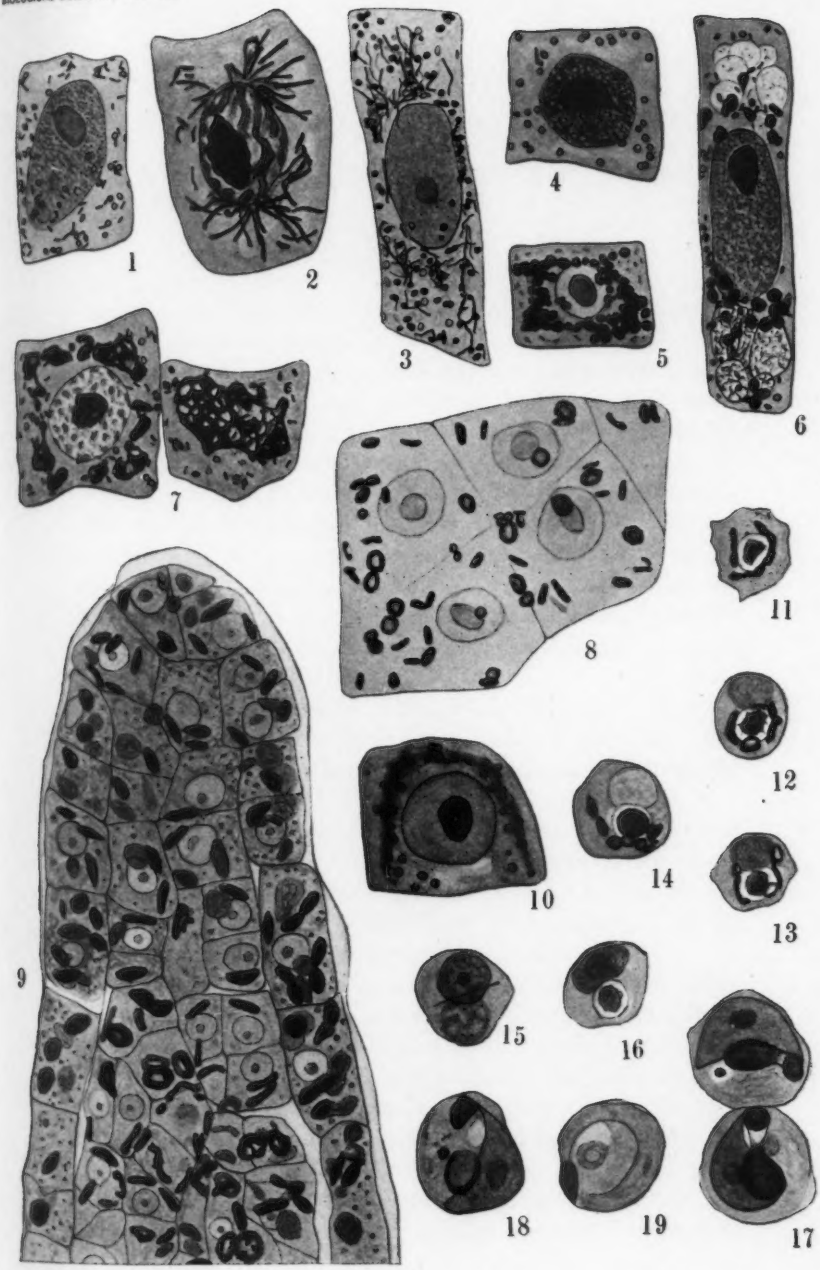
FIG. 10. Early androgonium. Champy-Fe-hematoxylin.

FIGS. 11 TO 14. Later phases of limosphere formation. Fig. 11 is viewed from one pole and does not show the nucleus. Kolatchev; Figs. 11 and 13 counterstained with acid fuchsin-aurantia.

FIGS. 15 AND 16. Centriole and tail filament in earlier sperm-forming stages. Benda.

FIGS. 17 AND 18. Stages in the deposition of the acrosomal material. The heavily stained periphery of the limosphere is now known to have little if anything to do with the osmiophilic platelets. Allen's fluid-Fe-hematoxylin.

FIG. 19. Early elongation of the acrosome. Benda.



R. H. BOWEN.

A PATHOGENIC LUMINESCENT BACTERIUM.

O. L. INMAN,

ANTIOCH COLLEGE.

In 1889 Giard and Billet (1) found that a number of different kinds of amphipod crustacea (*Talitrus*, *Orchestia*, *Ligia*, and others) gave off light. This luminosity was found to be caused by the presence of luminous bacteria in the tissues of the crustacea. In working with these luminous bacteria Giard and Billet were able to transfer them from one marine crustacean to another with ease and when grown on any marine crustacean tried, the bacteria became luminous. The authors could not get the bacteria to luminesce upon artificial media, although they obtained good growth. They offered no explanation for this failure and Harvey (2) pointed out that it seemed peculiar that these organisms could not be grown on artificial media so that they would produce light.

The luminous bacterium isolated by Giard and Billet is perhaps best known as *Bacterium Giardi* (Kruse) Mig., but much work is still needed upon the classification of luminous bacteria.

In August, 1925, several luminous individuals of *Orchestia platensis* (Kreyer) and *Talorchestia longicornus* (Say) were brought into the laboratory of E. N. Harvey at the Marine Biological Laboratory, Woods Hole, Massachusetts, and he turned them over to the author for investigation.

The sand flea chiefly studied was *Talorchestia longicornus* (Say) which can be found buried in the sand just above the high tide line during the daytime and feeding at the water's edge at night. This species is about the size of a honey bee and while they can be collected in June no luminous individuals have so far been found until August. These sand fleas will live several days in the laboratory if left in dishes of moist sand.

When luminous sand fleas were broken open and the grayish colored exudate mounted under the high power of a microscope,

numerous small motile rod-shaped bacteria could be seen. Inoculation of some of this material from the sand flea upon sea water peptone agar to which had been added sufficient sodium hydroxide to bring the pH to 8.1 resulted in the appearance of numerous bluish-green luminous colonies of bacteria in from twenty-four to thirty-six hours. These colonies were picked and pure cultures obtained. By a series of transfers it was readily determined that this bacterium in pure culture growing upon artificial media of many different kinds would produce light if the proper osmotic pressure and hydrogen ion concentration were maintained. The optimum, minimum, and maximum salt concentrations and hydrogen ion concentrations for growth and light production have not been determined but sufficient evidence is at hand to show that growth and light production may take place in a wide concentration of salts and hydrogen ion concentrations, the greater range from sea water being toward fresh water and neutrality. Data on this phase of the subject will be published in another paper.

Thus it at once became evident that Giard and Billet were unable to obtain luminous organisms upon artificial media because they failed to adjust the hydrogen ion concentration of their media which became neutral to acid when they added peptones to sea water and in such a case good growth may be obtained but no luminescence.

Giard and Billet also did sufficient experimentation to satisfy themselves that this bacterium was pathogenic to sand fleas. They inoculated sand fleas and followed the cases through to the death of the organisms which they claimed occurred more quickly than normal sand fleas will die in the laboratory.

The author repeated some of this work and found that it was possible to transfer the bacteria quite easily from one crustacean to another and that the administration of large numbers of this bacterium to a sand flea did cause it to die sooner than normal fleas. There were cases, however, when transfer of bacteria or feeding bacteria seemed to have doubtful effect and the sand fleas failed to become luminous. This led the author to collect non-luminous sand fleas from various habitats and isolate bacteria from the intestinal tract. In

almost every one of twenty separate sand fleas tried, luminous bacteria could be isolated and grew well and produced light on artificial media. The luminous bacteria isolated were quite surely *Bacterium Giardi*, but more proof is needed to demonstrate that this is the only species of luminous bacteria which inhabits the sand flea. This demonstrates, as pointed out by Dahlgren (3), that these bacteria are not necessarily luminous continuously, although it does not preclude the fact that they may be kept continually luminous without being passed through the sand flea, as has now been done for two years by the author. This observation also seems to point to the conclusion that this bacterium is a normal inhabitant of the body of the sand flea but that at certain times and under certain conditions it invades the muscles and increases in number so rapidly that the sand flea becomes luminous and dies. It is possible that the bacteria do not become luminous until they have spread to the muscular tissue of the sand flea and thus luminosity would mean that the host was doomed to die, since I have never observed a luminous sand flea recover. As to what causes the bacteria to invade the muscular tissue, especially in August at Woods Hole, Massachusetts, nothing yet is known. It may be a lowered resistance of the sand flea or increased reproductive activity of the bacterium due to a higher temperature or due to other causes. It should also be said that out of examination of possibly twenty thousand sand fleas only a very few ever become luminescent and it is quite possible that this bacterium plays a part in the death of thousands of sand fleas that never become luminescent. In other words, if this bacterium is really pathogenic, of which there is still some doubt, it is doubtful if there is any connection between luminosity and pathogenicity other than that great numbers of this bacterium under favorable environmental conditions naturally produce a maximum deleterious effect upon sand fleas and also give off more light.

Summary.

Amphipod crustacea are the host of *Bacterium Giardi* which becomes luminous under certain conditions and may kill the sand flea.

This bacterium, if isolated in pure culture and grown upon peptone sea water agar of pH 8.1, becomes luminous within twenty-four hours and may be kept so by frequent transfer for at least two years.

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SOME FACTORS AFFECTING THE TOXICITY OF HYDROCYANIC ACID FOR INSECTS.

F. J. BRINLEY AND R. H. BAKER,[1]

ZOÖLOGICAL LABORATORY, UNIVERSITY OF PENNSYLVANIA.

During the last twenty years a vast amount of work has been done on the control of injurious insects by the use of hydrocyanic acid. Inasmuch as little quantitative work seems to have been done on the influence of environmental factors, such as temperature and humidity, upon the toxicity of HCN to insects, and since the relation of concentration of cyanide to length of exposure also does not seem to have been thoroughly investigated, it was thought advisable to study these points in some detail.

APPARATUS AND METHOD.

To determine the effect of concentration of cyanide, length of exposure, temperature and humidity upon the toxicity of HCN to insects, apparatus by which all these factors can be controlled or varied must be employed. In the present experiments, such apparatus was devised and proved to be very satisfactory.[2]

The apparatus, as shown in Fig. 1, consists of the following units: (*A*) flow regulator (one-inch glass tube 5 feet long); (*B*) humidifying unit, consisting of two half-liter bottles; (*C*) two flow meters; (*D*) generating flask; (*E*) half-liter bottle used as a fumigation chamber, placed in a water bath; (*F*) a small absorption bottle used for collecting samples of gas for analysis; (*G*) a one-liter bottle filled with NaOH for absorbing the gas. The outlet at H is attached to a suction pump.

The air flows in the direction indicated by the arrow. The rate of flow can be regulated by raising or lowering the glass tube

[1] This work was done in coöperation with the American Cyanamid Sales Company.

[2] The writers wish to acknowledge the assistance given by Mr. K. D. Ashley, of the Chemical Staff of the American Cyanamid Sales Company, who furnished valuable suggestions for devising the apparatus and for the colloidal silver iodide method of analysis.

(*a*). The humidifying unit is filled with either distilled water or various strengths of sulfuric acid to give the desired humidity. The air, after passing through the flow regulator, passes over the humidifying unit and through the first flow meter, which gives

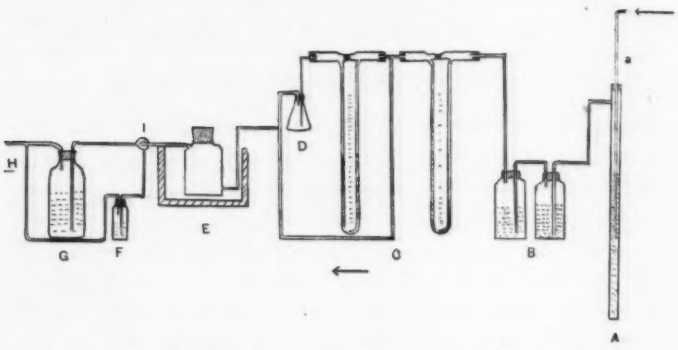

FIG. 1. Apparatus used in determining the toxicity of HCN to insects.

the total rate of flow in liters per hour. The air line is divided after leaving flow meter No. 1; part of the air passes directly into the gassing chamber *E*, the remainder of the air goes through the second flow meter, thence to the generating flask *D*, which contains the source of HCN (calcium cyanide or liquid HCN). The air saturated with HCN mixes with the pure air in the tube leading to the gassing chamber *E*. The apparatus is so designed that the concentration of HCN can be regulated by controlling the amount of air which passes through the second flow meter. The reading of the second meter gives the rate of flow of air through the generating flask. The air-gas mixture, after leaving the gassing chamber, passes into the absorption bottle *G* containing a concentrated solution of NaOH for the purpose of absorbing the HCN, thus preventing any escape of the gas into the room.

To collect a sample of gas for analysis, the gas-air mixture is passed through a small absorption bottle *F* by turning a two-way stopcock *I*. In this work two methods of analysis were used. The first method consists of collecting one liter of gas in a 10 per cent. NaOH solution and titrating with silver nitrate.

By the second method the HCN is passed into an alkaline, colloidal solution of silver iodide, which is made by adding potassium iodide to a known normality of silver nitrate. The time which is required to decolorize the solution is taken by means of a stopwatch. The first method of analysis, is perhaps, more accurate, but as it requires more time than the second, it was used only as a check on the AgI method. By the use of the second method, several samples could be tested during one exposure, so that accurate record could be kept of the concentration of HCN.

The insects used in the tests were kept in a container in a water bath maintained at a desired temperature for 1 hour before fumigating and the fumigation chamber was kept at the same temperature. After fumigating the insects, they were replaced in the water bath until the following morning, when they were removed and the mortality was determined. Throughout this work, unless otherwise stated, granular calcium cyanide was used as a source of HCN.

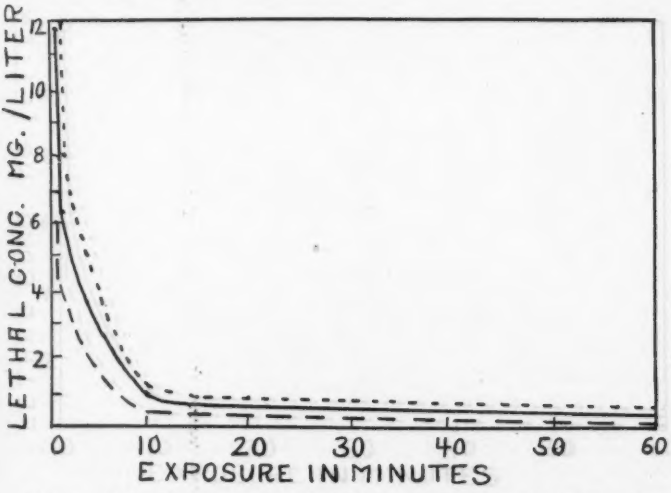

FIG. 2. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Macrosiponiella sanborni*. Solid line, 25° C.; dotted line, 20° C.; broken line, 30° C.

Relation of Concentration of HCN to the Length of Exposure.

Experiments were carried out to determine whether a low concentration of cyanide for a long period of time was more fatal than a high concentration for a short period of exposure. When using $Ca(CN)_2$ under field conditions, where one is not able to obtain a long period of exposure, it is necessary to use a very high concentration.

The results obtained from more than a thousand tests with two species of aphids and one species of thrips, show that concentration and length of exposure are inversely related up to a certain time, *i.e.*, toxicity = concentration × the time. Beyond a period of 15 minutes it seems that concentration is independent of the time, as it takes practically the same concentration to kill in one hour as in 30 minutes (Figs. 2, 3 and 4).

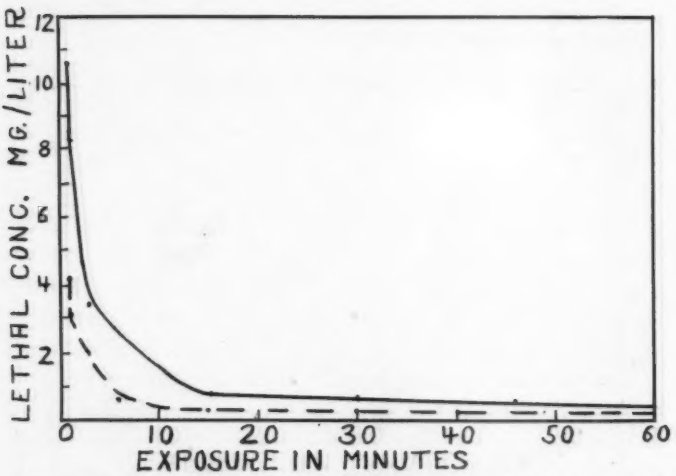

Fig. 3. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Aphis rumicis*. Solid line, 25° C.; broken line, 30° C.

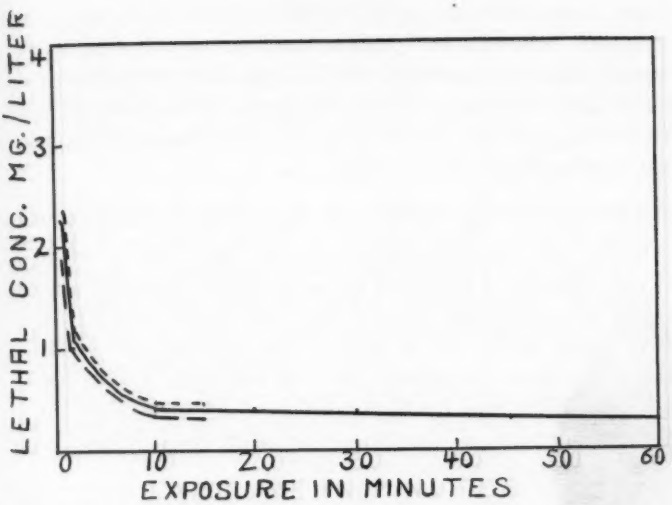

FIG. 4. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Thrips tabaci*. Solid line 25° C.; broken line, 30° C.; dotted line, 20° C.

TEMPERATURE.

It is known that metabolic processes increase with increase in temperature. With an increase in metabolic activities, we would expect an increase in the susceptibility of the insect to HCN. That such appears to be the case is shown in Figs. 2, 3 and 4. It may be noted from these figures that the effect of temperature is more pronounced with the shorter exposures.

Throughout the experiments on aphids, it was noted that the young organisms and the winged adults were more susceptible than the wingless adults. These results coincide with Child's [1] idea that the physiologically young organisms have a higher rate of metabolism than the older animals and are more susceptible to cyanide. In the case of thrips, however, the adults seem to be easier to kill than the young.

[1] Child, "Senescence and Rejuvenescence," Chicago University Press, 1915.

Humidity.

Several humidity experiments were conducted using *Aphis rumicis*, *Sitophilus granarius* and *S. oryza* under various relative humidities, other factors being the same. The result of these experiments indicate that humidity is not an important factor in determining the toxicity of HCN.

Comparison of the Toxicity of Liquid HCN and $Ca(CN)_2$.

During the course of the experiment, the question arose as to the comparative toxicity of hydrocyanic acid resulting from the action of water vapor on $Ca(CN)_2$ and the HCN volatilizing from liquid HCN. It is known that in addition to HCN, there are other compounds, such as ammonia and hydrogen sulphide, which arise from hydrolysis of $Ca(CN)_2$, and so it was thought advisable to ascertain what effect these foreign gases would have upon the toxicity of HCN.

The results indicate that the pure HCN coming from liquid HCN is slightly more toxic than the gas mixture which arises from $Ca(CN)_2$. These results are also confirmed in a number of experiments by adding slight quantities of NH_3 and H_2S to the gas which arises from liquid HCN, showing that there is some antagonistic action between the ammonia, hydrogen sulphide and HCN, thus lowering the toxicity of HCN.

Effect of Methyl Acetate on the Toxicity of HCN.

Some insects, such as *Melanoplus differentialis*, when placed in a sublethal concentration of HCN, close their spiracles and thus prevent, to a certain extent, the entrance of the cyanide gas.

It was thought that if some chemical could be mixed with the HCN, which would keep the spiracles open, the insects would be more easily killed by cyanide than if the spiracles are allowed to close. Methyl acetate is found to keep the spiracles open in *M. differentialis* when the insect is placed in an atmosphere of HCN. Experiments were conducted to determine what effect the addition of a small amount of methyl acetate would have upon the toxicity of pure HCN. The only insects available at the time of the experiments were *S. granarius* (grain beetle),

and it was not determined whether or not the insects close their spiracles. However, when the methyl acetate was added to liquid HCN, the average kill as shown from ten experiments was 77.4 per cent. as compared with a 59 per cent. kill when pure HCN was used.

SUMMARY.

A detailed study was made of the toxicity of HCN for certain insects. The insects used in this work were two species of aphids, *Aphis rumicis* and *Macrosiphoniella sanborni*, one species of thrips, *Thrips tabaci*, and two species of grain beetles, *Sitophilus granarius* and *S. oryza*. Apparatus by which all factors can be controlled or varied was employed.

The results of over a thousand experiments indicate that, within certain limits, concentration and length of exposure are inversely related or that toxicity = concentration × the time.

The higher the temperature, the more susceptible were the insects; this susceptibility was more pronounced with the shorter exposures.

Present indications are that humidity is not an important factor affecting the toxicity of HCN. Comparative studies on calcium cyanide and liquid cyanide show that the liquid HCN is more toxic than the gases from hydrolysis of calcium cyanide. A small amount of methyl acetate added to liquid HCN seemed to increase the toxicity of the gas arising from the liquid HCN, which may be due to the fact that a small amount of methyl acetate kept the spiracles open, while in pure HCN the spiracles were quickly closed.

THE NATURAL HISTORY OF *CUMINGIA TELLINOIDES.*

BENJAMIN H. GRAVE,[1]

WABASH COLLEGE (SEESSEL FELLOW 1926–27).

INTRODUCTION.

When it is considered that *Cumingia* is used extensively as a source of material for embryological and experimental studies it becomes a matter of importance to know its life history and its availability at all times. An observation made several years ago indicated that *Cumingia* exhibits a lunar periodicity in spawning and led the writer to make an extended study of the species covering a continuous period of five years. The laboratory work was carried on at the Marine Biological Laboratory at Woods Hole.

With the perfecting of the diatom method of feeding embryos it is possible to carry many marine larvæ through metamorphosis and thereby gain a knowledge of complete life histories where only fragmentary knowledge has been possible before. In this instance a pure culture of the diatom Nitschia, which was isolated at the Plymouth Laboratory, was used.[2] The diatoms at Woods Hole are not suitable for feeding because of the danger of bacterial infection of the embryos. The Beaufort species first used by Caswell Grave, on the other hand, grows in great abundance in aquarium jars and may be fed without difficulty. This diatom has the advantage over Nitschia in being relatively short and thick instead of long and slender. Both of these species should be propagated at marine laboratories for the use of investigators.

HABITAT.

Cumingia tellinoides is found principally in sandy bottoms containing a strong admixture of humus, with a sparse or moderate

[1] From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn., and the Marine Biological Laboratory, Woods Hole, Massachusetts.

[2] The writer wishes to thank Dr. Isabella Gordon for a stock of this material.

growth of eel grass, and where the tidal currents run swiftly as in narrow channels. The vertical distribution is not great, being a little above and below low tide mark.

The animal is readily dug with a spade at low tide and separated from the dirt with a coarse sieve. Under favorable conditions one can collect three or four dozen in the course of an hour. It has long siphons which enable it to lie inches below the surface of the bottom and yet feed from the water in the manner of lamellibranch mollusks generally, the food being chiefly diatoms and floating particles of organic matter. It is rather particular about the kind of bottom and the nature of the water currents and is therefore restricted in its distribution.

The Spawning Season.

Cumingia begins to spawn about the middle of June and continues until the middle of September. One can usually depend upon getting eggs in abundance until about August 20. At that time there is likely to be a lull or often a complete cessation in spawning until the first of September or until the next full moon. Eggs can usually be obtained in considerable quantity during the first half of September. Not all individuals are spawning in September and few are spawning heavily as a rule. Each adult spawns more than once during the season and the production of gametes is apparently continuous.

Method of Securing Eggs.

Cumingia spawn very readily in the laboratory but it is necessary to treat them according to schedule. Experience has shown that the best way to obtain clean eggs or sperm is to wash the animals free from all sediment and isolate them in small stender dishes half filled with sea water. When so placed in sea water and left undisturbed for an hour or less they extend their siphons and spawn if sexually mature. The eggs or sperm are thrown forcibly from the dorsal siphon as they accumulate in the supra-branchial chambers. The sexes are separate and may be distinguished by their color. The males are white and the females pink. The shell is not heavy so that the color of the gonads shows through more or less.

Cumingia cannot be kept in the laboratory indefinitely. It is desirable to use them the day they are collected, although it is sometimes possible to keep them for a day or two in moist sand. They must be kept away from water if it is desirable to have them retain their eggs or sperm.

The Egg. Size, Structure, Membranes.

The egg of *Cumingia* measures in extreme limits from .061 mm. to .065 mm. while the usual size is .062 or .063 mm. In color it varies from a light gray to a distinct pink. The animal pole of the egg is comparatively free from yolk and pigment so that it appears light in color. Centrifuged eggs show a beautiful banding due to the separation of materials of different specific gravity. These materials consist of yolk, pigment, oil and clear protoplasm.

The polarity of the egg is fairly rigidly fixed as shown by the fact that in centrifuged eggs the cleavage planes remain unchanged although the pigment and so-called formative materials may be driven to any part of the egg. This general problem was investigated by Morgan in 1910 and the phase of it referred to here was fully verified by myself in 1924. He was concerned with the influence of the "organ-forming substances" upon development.

Surrounding the egg there is a jelly membrane about .03 mm. in thickness or half the diameter of the egg. It is wholly invisible under the microscope but readily demonstrable by the use of a suspension of india ink in the water. One might suspect the presence of this jelly membrane by the circumstance that the eggs in a dish do not come into contact but are separated by a little space. This tertiary membrane has apparently been overlooked by investigators. There is also a distinct vitelline membrane which undergoes little or no visible change after fertilization. The membrane is not perceptibly lifted after the penetration of the spermatozoön so it may be proper to say that there is no fertilization membrane. This may account for the fact that the *Cumingia* egg is at the mercy of the sperm and polyspermy is common. At any rate it is desirable to learn whether this susceptibility to polyspermy is due to absence of one

or more of the egg secretions that Lillie has shown to be concerned with the fertilization reaction in the Nereis egg, and which sweep supernumerary spermatozoa from the surface of the egg after the initiation of the fertilization reaction. It frequently happens that one can count ten or a dozen sperm nuclei within an egg that has been too heavily inseminated. Cases have been seen in which every egg showed polyspermy of this extreme character. When eggs are spawned the germinal vesicle has already broken down and the first polar spindle has formed. On this account the first polar body appears very promptly after insemination.

Morgan found that the egg of *Cumingia* is very easily injured by rough handling so that it may fail to develop normally beyond cleavage. At first he was unable to secure normal veliger larvæ from centrifuged eggs. He finally centrifuged the entire animal before it had spawned and, to his surprise, it spawned completely centrifuged eggs which developed normally, showing that the dislocation of the egg materials did not interfere with normal development. He came to the conclusion that the trouble he had experienced was due to injury to the membranes of the egg.

Rate of Growth, Metamorphosis and Life History.

The polar body is of interest chiefly because of its great size and mode of formation. It seems to round up before it comes to the surface of the egg and to migrate out, breaking its way through the cytoplasm of the egg as the latter is pushed up into a rounded prominence. The vitelline membrane, being thick and elastic, is pushed up locally. As a curious anomaly, no doubt associated with polyspermy, one case was observed in which two polar bodies came off at the same time, side by side, and were followed after the usual interval by a third polar body. As a rule only two polar bodies are formed. The first one rarely divides, although its division has been observed now and then. In case of polyspermy the cell may go into the three-celled condition at the first cleavage, and the polyspermic embryos may be normal or abnormal. *Cumingia* would, in fact, be first class material for a study such as Boveri's on the Echinoderm egg.

The rate of development and age at metamorphosis are shown in Table I. The later stages were obtained by feeding the

TABLE I.

SCHEDULE OF NORMAL DEVELOPMENT AT MEDIUM SUMMER TEMPERATURES, 20°–21° C.

First polar body	12 to 15 minutes
Second polar body	28 to 35 "
First cleavage	60 to 70 "
Second cleavage	90 to 102 "
Blastula	5 to 6 hours
Gastrula	7 to 10 "
Trochophore	12 to 18 "
Metamorphosing trochophore	20 to 30 "
Veliger complete	2 to 24 days
Metamorphosis of veliger into adult form	16 to 24 "
Sexually mature in one year	
Fully grown in four years	
Duration of life two to four years	

larvæ, by means of which they were kept alive for a month. One can distinguish the new parts of the shell, as added to the original larval shell, and so tell where new growth has occurred. It is quite likely that the development of the larvæ in the open ocean is more rapid and uniform than in the laboratory. However, the veligers seemed to thrive upon diatoms in a large aquarium jar kept agitated by a mechanical device, and in time a considerable number went through metamorphosis.

The measurements given in Table III. show that the veligers grew somewhat before metamorphosis but more rapidly afterwards. This is not fully in line with data on the rate of growth of *Chiton* (unpublished), which show that there is no measurable growth until after metamorphosis. *Chiton* seems to be unusual in this respect. The larvæ of *Cumingia* did not develop uniformly in this aquarium habitat. Some grew symmetrically, adding equally to the shell all around, and others grew asymmetrically. I cannot, therefore, be sure of the normal form. Moreover, there is very considerable variation in the proportions of the normal veligers of a single female. If variation is as extensive in all lamellibranchs as in *Cumingia* it is difficult to see how the early larvæ of different species can ever be distinguished satisfactorily by measurements, although Stafford has

been able to recognize the older larvæ. The chief difference that I have observed between the young veligers of various lamellibranchs is in their general appearance. Some have a thicker shell than others and therefore refract light differently. As is well known the veligers of all lamellibranchs are so much alike that they can be distinguished, if at all, only by experts. The differences are largely indefinable and are apparent only after long study.

TABLE II.

SCHEDULE OF DEVELOPMENT AT HIGHER SUMMER TEMPERATURES, 22°–23° C.

First polar body	7 to 9 minutes
Second polar body	27 to 30 "
First cleavage	45 to 50 "
Second cleavage	70 to 75 "

Eggs developing at the rate shown in Table II. may reach the complete veliger stage in from twenty-four to thirty hours. The rate of development which Morgan gives for *Cumingia* trochophores and veligers is considerably too slow, and his statement that unfertilized centrifuged eggs are intact after forty-eight hours is also erroneous. Unfertilized eggs fragment within twenty-four hours. It should be noted, however, that he was not particularly concerned with the rate of development and these oversights do not affect the validity of the conclusions he draws.

TABLE III.

MEASUREMENTS AT VARIOUS AGES.

Egg .061 to .065 mm.—average .062 to .063.
Blastula same as above.
Trochophore same as above but slightly elongated anteroposteriorly.
Two day veliger .074 x .090 x .055 mm. to .08 x .091 x .055 mm.
Ten day veliger .09 x .10 mm.
Two weeks veliger .095 x .105 mm.
Three weeks Cumingia metamorphosed .09 x .105 mm. to .097 x .155 mm.
Four to five months (size attained before first winter.), 6 x 5 mm. to 10 x 8 mm.
One year, 11 x 8 mm. to 13 x 9 mm. (sexually mature).
Two years, 14 x 11 mm. to 16 x 11 mm.
Three years, 17 x 12 mm. to 17 x 13 mm.
Four years, 18 x 12 mm. to 19.5 x 14 mm. (Largest specimens found).

Experiments are in progress to learn the normal length of life of *Cumingia*. It appears that they are approximately half

RATE OF DEVELOPMENT AT VARIOUS TEMPERATURES.

Date.	Temp.	1st Polar Body.	2d Polar Body.	1st Cleavage.	2d Cleavage.
Aug. 20	18° C.	12–15 min.	40–45 min.	100–107 min.	150–160 min.
Aug. 20	18° C.	13–16 min.	50–55 min.	105–115 min.	154–165 min.
Aug. 21 (Two lots)	20° C.	*A* 13 min. *B* 12 min.	33 min. 32 min.	71 min. 68 min.	103 min. 101 min.
Aug. 20 (Two lots)	20° C.	*A* 10–13 min. *B* 11–13 min.	30–32 min. 31–33 min.	68–72 min. 69–73 min.	101–105 min. 102–105 min.
Aug. 18	20° C.	13–15 min.	33–35 min.	67–70 min.	100–105 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	73 min.
Aug. 10	22° C.	10 min.	33 min.	50 min.	75 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	72 min.
Aug. 18	29° C.	7–8 min.	20–22 min.	42–45 min.	62–67 min.
Aug. 21	30° C.	7–8 min.	19–20 min.	35–37 min.	54–57 min.
Aug. 28	33° C.	5 min.	20 min.	38–41 min.	58–61 min.
Aug. 20	35° C.	Tried three lots of eggs. None developed successfully. A few put off polar bodies and a few cleaved irregularly: one or two cleavages only.			

Note: These data show a rapid increase in rate of development from 18° up to 22° C. They do not show a proportionate increase in rate above that. 33° C. shows injury and irregularity. Eggs refused to cleave at 35° C.

It is only fair to say that this table should not be used for the calculation of temperature coefficients because the temperature was not kept rigidly uniform during the course of the experiments. It is sufficiently accurate for the purposes of this paper. It is possible to recognize end points to the minute although not all eggs cleave at the same instant as is indicated here.

grown and sexually mature in one year. I have the impression that they do not survive many years. Rings of growth on the shell show that they usually survive from two to four years, but the amount of growth after the third year is negligible.

In addition to the summarized data on the rate of development given in Tables I. and II. which are averages, I give the record of actual experiments in Table IV. It will be noted that the rates of development of several lots of eggs at temperatures ranging from 18° to 35° C. are given. It is interesting that the increase in rate of development from 18° to 23° C. is much greater than it is from 23° to 30° C.

The summer temperatures of the water at Woods Hole range from 18° to 22° C., the lower temperatures being those of June and late September, the higher being attained in July and August. The optimum temperature for the development of the *Cumingia* egg appears to be around 25° C. although the egg never experiences this temperature in nature.

The difference in rate of development of embryos between 18° and 25° is truly remarkable. One therefore does not speak of the normal rate of development of an embryo without considering the temperature. Because of certain interesting features of this table the writer expects later to describe more in detail the temperature coefficient of development of cleaving eggs.

Polyspermy.

Eggs spawned according to the method here described are convenient for study because they can be artificially fertilized at will. It is important to use only a drop of concentrated sperm suspension to fertilize a small stender dish of eggs, (in about 40 cc. of water) otherwise polyspermy results and abnormal development follows. As stated above, the *Cumingia* egg is particularly susceptible to polyspermy and it might be a legitimate problem for students of fertilization to undertake to explain why this is so, giving due consideration to Lillie's suggestion, *Jour. Exp. Zoöl.*, Vol. 14, 1913.

The Longevity of the Gametes.

If *Cumingia* eggs are allowed to remain unfertilized they ultimately die and disintegrate, though it has been found by

experiment that they vary greatly in their longevity. The eggs of some individuals are much more vigorous and survive for longer periods than those of others. The poorest lots of eggs fragment in from six to nine hours, and the best lots remain apparently normal from fifteen to twenty-two hours. The average lot of eggs begins to fragment at the end of nine or ten hours and 90 per cent. of them have fragmented after fifteen hours. We may say therefore that the average longevity of the unfertilized egg, during which normal fertilization is possible, is 10 to 12 hours. When using eggs older than these one may look for a considerable percentage of abnormal embryos. It is only the best lots of eggs which give normal development of all embryos when more than ten or twelve hours old although early cleavage may appear normal.

The average life of the sperm in the most dilute suspensions capable of giving 100 per cent. fertilization (1/6000 to 1/8000%) is 4 to 5 hours. The extremes are 3½ to 18 hours. If fresh eggs are added to these dilute sperm suspensions after nine or ten hours from forty to fifty per cent. of fertilizations often occur. On the average one can expect only 20 to 40 per cent. of fertilizations after nine hours and sometimes none at all. After ten hours they may fertilize from one to ten per cent. of the eggs. It was noted that abundant normal larvae developed from eggs that were fertilized by these aging spermatozoa up to nine or ten hours.

A sperm suspension of 1/500 to 1/750 % lives longer than the more dilute suspensions mentioned above. They may give 100% fertilization up to 9 or 10 hours and a considerable percentage of the spermatozoa may live for 12 hours. It was observed that they usually fertilize from one to five per cent. of the eggs from fifteen to twenty five hours. It is therefore apparent that the conditions existing in a concentrated sperm suspension are not the same as in more dilute suspensions.

The evidence goes to show that half of the spermatozoa that are shed into sea water under natural conditions die within five or six hours and that nine tenths of them die within ten hours. After a few hours most of the spermatozoa stop swimming and lie quietly except for a few lashings of the tail at intervals. In

the presence of eggs they become greatly activated and again swim vigorously. A very few are capable of becoming so activated up to the time when the life of the suspension becomes extinct, and a few fertilizations result, (1 to 5 per cent.).

It is interesting to note that J. Nelson observed that oyster sperm in the presence of egg secretions would wear out their limited store of energy within an hour and become incapable of causing fertilization.

I have not observed that spermatozoa in very dilute suspensions quickly lose their power to fertilize eggs while still motile as described by Professor Lillie for *Nereis* sperm, but it was observed that in very dense suspensions spermatozoa retain their vitality and ability to fertilize from two to three days. This phenomenon has been observed also by Gemmil, Lillie and Cohen. Gemmil ascribes it to the nutritive quality of the spermatic fluid, while Lillie and Cohen show that "it is due, at least in part, to the inhibiting effect of CO_2 in solution or to hydrogen ion concentration." Of course this extreme condition is never met with in nature.

Behavior of the Larva.

The larva becomes ciliated as a late blastula and, as an early gastrula it develops a strong prototroch and rises to the top. It swims vigorously until the trochophore fully transforms to the veliger. After becoming a typical veliger it soon settles to the bottom temporarily and from that time on it swims less and less frequently until it approaches the time of metamorphosis. Late veligers rarely swim and the velum appears to become more and more an accessory feeding organ. A certain amount of feeble movement is maintained until metamorphosis is completed, and the velum disappears. The velum persists until the form of the larva indicates that metamorphosis is practically complete though it becomes less and less vigorous after the first few days of active swimming.

Lunar Periodicity.

As indicated by the foregoing statements *Cumingia* shows a lunar rhythm in spawning. The period of the first quarter is the period of restricted spawning and full moon until new moon

or until nearly first quarter is the period of free spawning. During the summer of 1922 *Cumingia* was not spawning from August 15 until September 1. On September 1 spawning revived and eggs were obtained in abundance for several days following. (September 5 was full moon in 1922.) The cessation in mid-August may be described as sudden and the recovery in September was equally striking. This complete break was observed by others who were experimenting with *Cumingia* eggs. Records of other years show that such a clear-cut periodicity has been observed before but is not demonstrable every year. There is, however, almost without exception, a reduced spawning at the time of the first quarter of the moon not only in August but in June and July as well. There can be little doubt, therefore, that we are dealing here with a true lunar periodicity. There is always a period of from one to five days during the season when eggs are not obtainable after which spawning is revised.

Summary.

The breeding season extends from the second week in June to the middle of September. The gametes are produced continuously and spawning occurs more than once. A rhythm is present which is interpreted to be a lunar periodicity.

The rate of embryonic development is variable but under usual conditions the larva metamorphoses in about three weeks. Growth during the first two years is comparatively rapid and average adult size is attained at the end of the second year. Fortunate individuals may continue to live and grow for four years. Sexual maturity is reached in one year and spawning is abundant although greater quantities of gametes are produced during the second year.

The egg of *Cumingia* is subject to polyspermy and abnormal development often occurs if too heavily inseminated. The unfertilized egg will retain its vitality for approximately nine to twelve hours during which it is capable of developing normally. The spermatozoa when set free in sea water usually die within four to nine hours, a few die within three hours and a few live for twelve or fifteen hours.

The *Cumingia* egg is favorable material for experimental study

in that it is obtainable in abundance, is readily fertilizable artificially, and it withstands laboratory manipulation without injury.

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